IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLUMBIA
Civil Division

BIOGEN IDEC MA INC.,
14 Cambridge Center
Cambridge, MA 02142

Plaintiff,

v.

HON. DAVID KAPPOS,
Under Secretary of Commerce for
Intellectual Property and Director of the
United States Patent and Trademark Office
Office of General Counsel,
United States Patent and Trademark Office
Madison Building East, Rm. 10B20
600 Dulany Street, Alexandria, VA 22314,

Defendant.

Civil Action No.: _____

COMPLAINT

Plaintiff Biogen Idec MA Inc. ("Biogen"), for its complaint against the Honorable David Kappos, states as follows:

NATURE OF THE ACTION

1. This is an action by Biogen, the assignee of United States Patent No. 7,531,174 ("the '174 patent"), seeking judgment, pursuant to 35 U.S.C. § 154(b)(4)(A), that the patent term adjustment for the '174 patent be changed from 210 days to 531 days.

2. This action arises under 35 U.S.C. § 154 and the Administrative Procedure Act, 5 U.S.C. §§ 701-706.

THE PARTIES

3. Plaintiff Biogen is a corporation organized under the laws of Massachusetts, having a principal place of business at 14 Cambridge Center, Cambridge, MA 02142.

4. Defendant David Kappos is the Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office ("PTO"). The Director is the head of the PTO and is responsible for superintending the performance of all duties required by law with respect to the granting and issuing of patents, and is designated by statute as the official responsible for determining the period of patent term adjustments under 35 U.S.C. § 154.

JURISDICTION AND VENUE

5. This Court has jurisdiction to hear this action and is authorized to issue the relief sought pursuant to 28 U.S.C. §§ 1331, 1338(a) and 1361, 35 U.S.C. § 154(b)(4)(A) and 5 U.S.C. §§ 701-706.

6. Venue is proper in this district court by virtue of 35 U.S.C. § 154(b)(4)(A).

7. This Complaint is being timely filed in accordance with 35 U.S.C. § 154(b)(4)(A).

BACKGROUND

8. Michele Sanicola-Nadel, Kevin P. Williams, Susan Gail Schiffer, and Paul Rayhorn are the inventors of U.S. Patent Application Serial Number 10/693,538 ("the '538 application"), entitled "Cripto Blocking Antibodies and Uses Thereof," which was

filed on October 23, 2003 and issued as the '174 patent on May 12, 2009. The '174 patent is attached as Exhibit A.

9. Plaintiff Biogen is the assignee of the '174 patent, as evidenced by the records in the PTO, and is the real party in interest in this case.

10. Section 154 of 35 U.S.C. requires that the Director of the PTO grant a patent term adjustment in accordance with the provisions of section 154(b), which set forth a "Guarantee of prompt Patent and Trademark Office responses" and a "Guarantee of no more than 3-year application pendency."

11. In calculating the patent term adjustment, the Director must take into account PTO delays under 35 U.S.C. § 154(b)(1)(A) and (B), any overlapping periods in the PTO delays under 35 U.S.C. § 154(b)(2)(A), any disclaimer of patent term by the applicant under 35 U.S.C. § 154(b)(2)(B), and any applicant delays under 35 U.S.C. § 154(b)(2)(C).

12. On May 12, 2009, the '174 patent issued with a patent term adjustment of 233 days, as reflected on the face of the patent (*see* Ex. A at 1). On October 20, 2009, the PTO issued a Certificate of Correction revising the patent term adjustment to 210 days (*see* Exhibit B, Certificate of Correction).

13. On July 10, 2009, Biogen timely filed an Application for Patent Term Adjustment Including Request for Reconsideration under 37 C.F.R. § 1.705(b) and (d) ("the Request for Reconsideration"), requesting that the '174 patent be granted a corrected final patent term adjustment of 531 days based on the patentee's calculation of delay under 35 U.S.C. § 1.54(b)(1)(A) and (B). A copy of the Request for Reconsideration and Statement Under 37 CFR § 1.702(b)(2) is attached as Exhibit C, and

is incorporated herein by reference. The Request for Reconsideration for a corrected final patent term adjustment of 531 days was not granted, as indicated in Decisions mailed by the PTO on July 27, 2009 and September 1, 2009 (attached as Exhibits D and E, respectively).

14. Under 35 U.S.C. § 154(b)(4)(A), "[a]n applicant dissatisfied with a determination made by the Director under paragraph (3) shall have remedy by a civil action against the Director filed in the United States District Court for the District of Columbia within 180 days after the grant of the patent. Chapter 7 of title 5 shall apply to such action."

15. This action against the Director is timely filed within the 180 day period after grant of the patent, under 35 U.S.C. § 1.54(b)(4)(A).

CLAIM FOR RELIEF

16. The allegations of paragraphs 1-15 are incorporated in this claim for relief as if fully set forth.

17. The patent term adjustment for the '174 patent, as determined by the Director under 35 U.S.C. § 154(b) and listed in the Certificate of Correction mailed from the PTO on October 20, 2009, is 210 days (*See* Ex. B, Certificate of Correction).

18. The Director's determination of the 210-day patent term adjustment is in error because the PTO did not properly calculate and allow an adjustment for the delay in issuance of the '174 patent as set forth in 35 U.S.C. § 154(b). As indicated in the "Statement Under 37 CFR § 1.702(b)(2)," the correct patent term adjustment for the '174 patent is 531 days (*see* Ex. C).

19. Biogen is entitled to an adjustment of the term of the '174 patent under 35 U.S.C. § 154(b)(1)(A) in the amount of 322 days, which is the number of days attributable to PTO examination delay during prosecution of the '538 application ("A Delay"). This 322 day period includes a delay of 299 days for failure by the PTO to mail an action under 35 U.S.C. § 132 not later than 14 months after the filing date of the application, and a delay of 23 days for failure by the PTO to issue a patent not later than four months after the date on which the issue fee was paid under 35 U.S.C. § 151.

20. Biogen is also entitled to an additional adjustment of the term of the '174 patent under 35 U.S.C. § 154(b)(1)(B) in the amount of 380 days, which is the number of days the issuance of the '174 patent was delayed beyond three years after the actual filing date of the '538 application ("B Delay"). This period is calculated beginning on the day after the date that is three years after the date on which the above-referenced patent was filed under 35 U.S.C. § 111(a) (*i.e.*, October 24, 2006), and ending on the date Patentees first filed a Request for Continued Examination (*i.e.*, November 8, 2007).

21. Section 35 U.S.C. § 154(b)(2)(A) states that "[t]o the extent that periods of delay attributable to grounds specified in paragraph [154(b)(1)] overlap, the period of any adjustment granted under this subsection shall not exceed the actual number of days the issuance of the patent was delayed." For the '174 patent, none of the A Delay overlapped with the period of B Delay. Therefore, there is no period of overlap to be excluded from the patent term adjustment.

22. The total period of PTO delay was 702 days, which is the sum of the A Delay (322 days) and the B Delay (380 days), as shown in the chart attached hereto as Exhibit F.

23. The '174 patent is not subject to a disclaimer of term. Therefore, the period of patent term adjustment is not limited under 35 U.S.C. § 154(b)(2)(B).

24. The total period of PTO delay is reduced under 35 U.S.C. § 154(b)(2)(C) by the period of applicant delay, which the Director calculated for the '174 patent as 171 days.

25. Accordingly, the correct patent term adjustment to which Biogen is entitled under 35 U.S.C. § 154(b)(1) and (2) is 531 days, which is the total period of PTO delay (322 days + 380 days= 702 days), less the period attributable to applicant delay (702 days - 171 days = 531 days).

26. The Director erred in the determination of patent term adjustment for the '174 patent by erroneously treating the entire period of B Delay as overlapping with the A Delay, and crediting only the greater of the two delays, instead of correctly crediting the sum of both the A and B delays. Thus, the Director incorrectly calculated a patent term adjustment for the '174 patent as 210 days (381 days – 171 days = 210 days).[1] By this erroneous calculation, the Director has deprived Biogen of the full patent term adjustment to which it is entitled (calculated above as 531 days).

27. In its opinion in *Wyeth v. Dudas*, 580 F.Supp.2d 138 (D.D.C. 2008), this Court explained the proper construction and application of the provisions of 35 U.S.C. § 154(b) for determining patent term adjustment. In accord with this Court's decision in

[1] Plaintiff notes that, in a decision mailed September 1, 2009 (*see* Ex. E), the Director recalculated and revised the patent term adjustment from 210 days to 233 days and indicated that the PTO intends to issue a second Certificate of Correction reflecting this latest change. At the time the instant Complaint was filed, however, the PTO had not yet issued the second Certificate of Correction. Nevertheless, when it does, the Director's revised patent term adjustment of 233 days will still be erroneous and contrary to the 531-day patent term adjustment to which Biogen is legally entitled.

Wyeth, the patent term adjustment for the '174 patent is properly determined to be 531 days, as set forth above.

28. The Director's allowance of only 210 days of patent term adjustment for the '174 patent is arbitrary, capricious, an abuse of discretion, and otherwise not in accordance with law and in excess of statutory jurisdiction, authority or limitation.

WHEREFORE, Plaintiff respectfully prays that this Court:

A. Issue an Order changing the period of patent term adjustment for the '174 patent term from 210 days to 531 days, and requiring the Director to alter the term of the '174 patent to reflect the 531 day patent term adjustment.

B. Grant such other and further relief as the nature of the case may admit or require and as may be just and equitable.

Respectfully Submitted,

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Dated: November 6, 2009

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EXHIBIT A

(12) **United States Patent**
Sanicola-Nadel et al.

(10) **Patent No.: US 7,531,174 B2**
(45) **Date of Patent: May 12, 2009**

(54) **CRIPTO BLOCKING ANTIBODIES AND USES THEREOF**

(75) Inventors: **Michele Sanicola-Nadel**, Winchester, MA (US); **Kevin P. Williams**, Chapel Hill, NC (US); **Susan Gail Schiffer**, Lexington, MA (US); Paul Rayhorn, Foxborough, MA (US)

(73) Assignee: Biogen Idec MA Inc., Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 233 days.

(21) Appl. No.: **10/693,538**

(22) Filed: **Oct. 23, 2003**

(65) **Prior Publication Data**

US 2004/0146940 A1 Jul. 29, 2004

Related U.S. Application Data

(63) Continuation of application No. PCT/US02/11950, filed on Apr. 17, 2002.

(60) Provisional application No. 60/367,002, filed on Mar. 22, 2002, provisional application No. 60/301,091, filed on Jun. 26, 2001, provisional application No. 60/293,020, filed on May 17, 2001, provisional application No. 60/286,782, filed on Apr. 26, 2001.

(51) **Int. Cl.**
A61K 39/395 (2006.01)
A61K 38/00 (2006.01)

(52) **U.S. Cl.** **424/141.1**; 424/130.1; 424/178.1; 424/277.1; 530/387.1

(58) **Field of Classification Search** 435/69.1, 435/320.1, 325, 252.3, 254.11, 69.6; 530/350, 530/300, 399, 387.1; 536/23.5, 23.51, 23.1; 424/141.1, 130.1, 178.1, 277.1
See application file for complete search history.

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(Continued)

Primary Examiner—Sheela J Huff
(74) *Attorney, Agent, or Firm*—Lahive & Cockfield, LLP; Megan E. Williams

(57) **ABSTRACT**

The invention provides Cripto blocking antibodies, or biologically functional fragments thereof, and uses thereof. Antibodies which bind Cripto and modulate Cripto signaling are provided. Antibodies which bind Cripto and block the interaction between Cripto and ALK4 are provided. Antibodies which bind Cripto and modulate tumor growth are also provided. Antibodies which bind Cripto, modulate signaling, and modulate tumor growth are also provided. Antibodies which bind Cripto, block the interaction between Cripto and ALK4 and modulate tumor growth are provided. The invention also provides methods of using these antibodies in therapeutic, diagnostic, and research applications.

228 Claims, 3 Drawing Sheets

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FIGURE 1

Response of the NCCIT, Human Testicular Carcinoma Cell Line, to anti-CFC blocking Cripto mAb A8G3.5

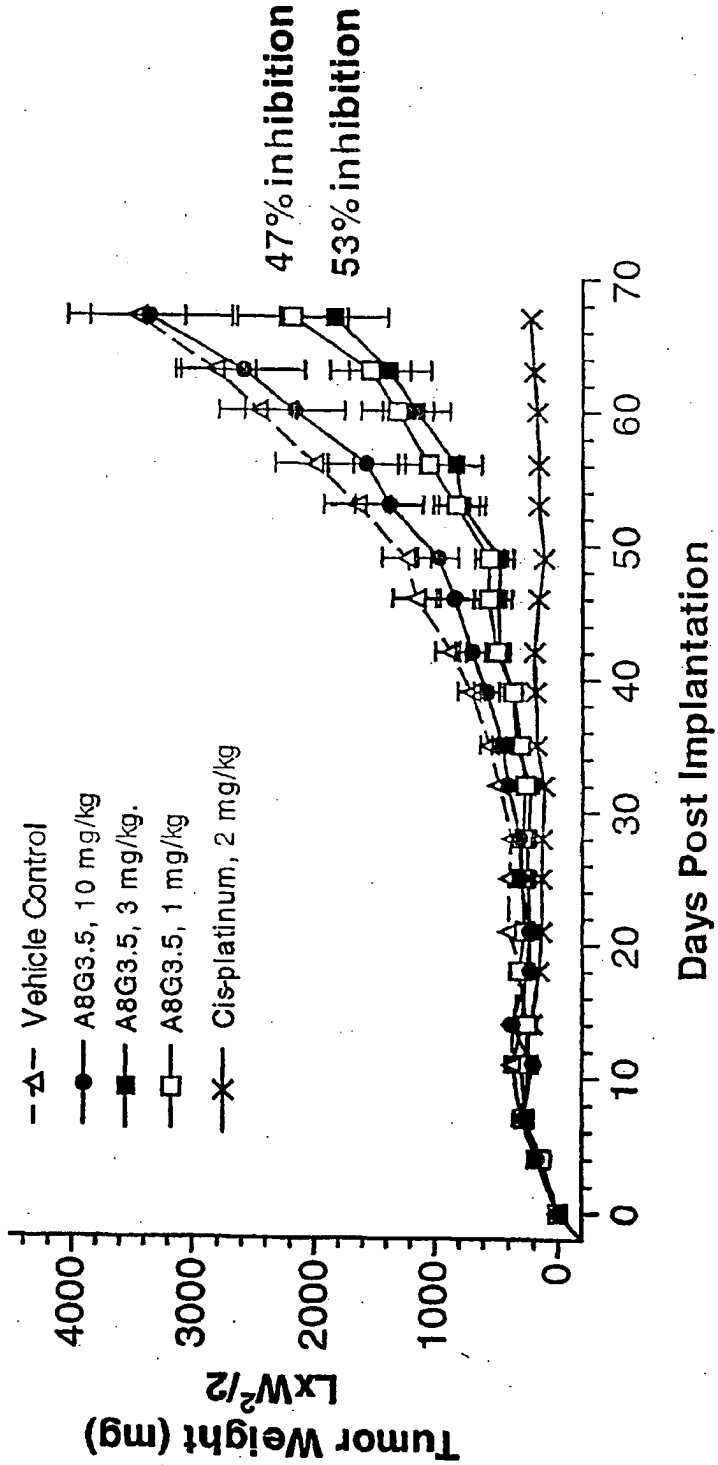


FIGURE 2

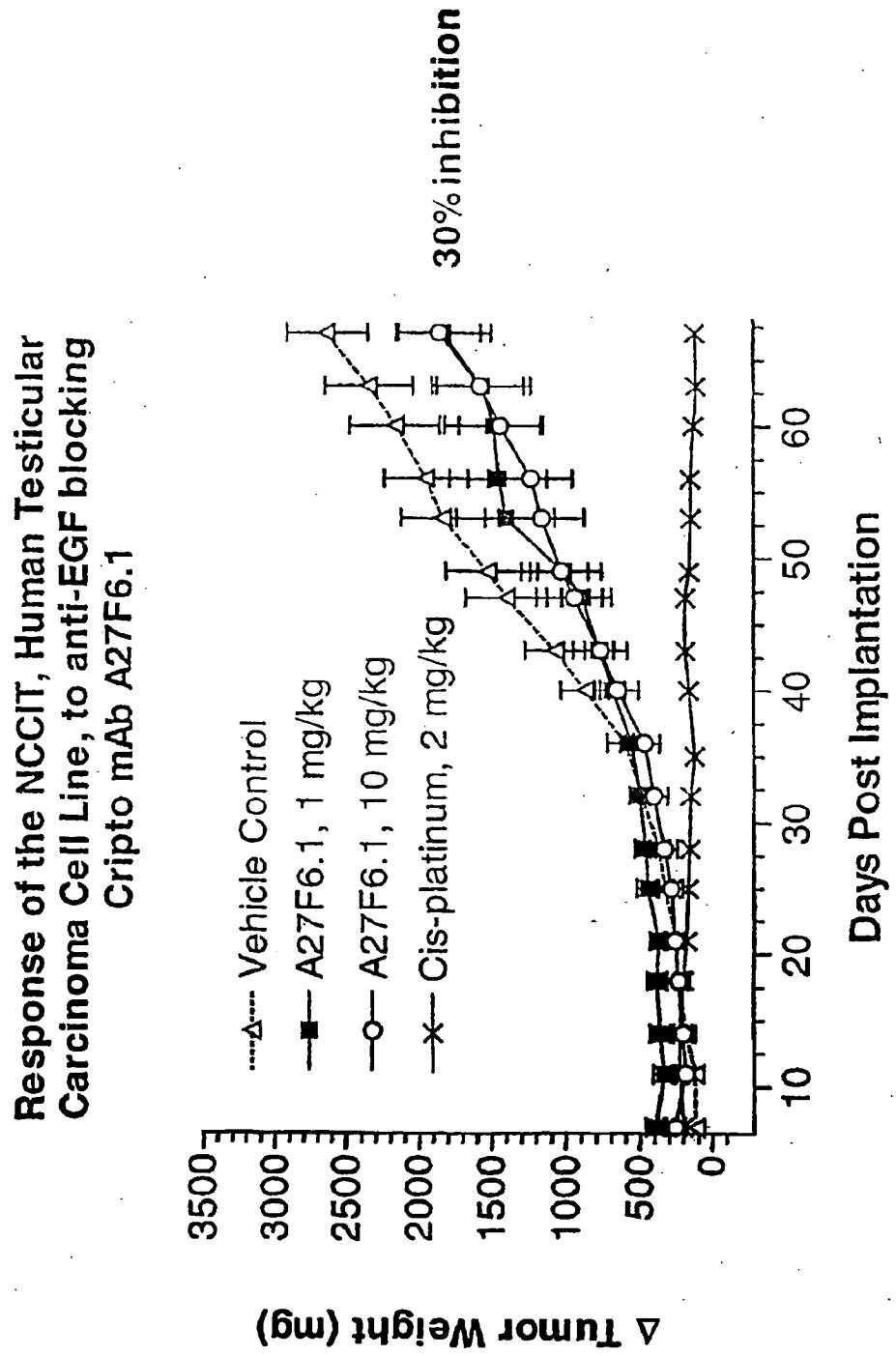
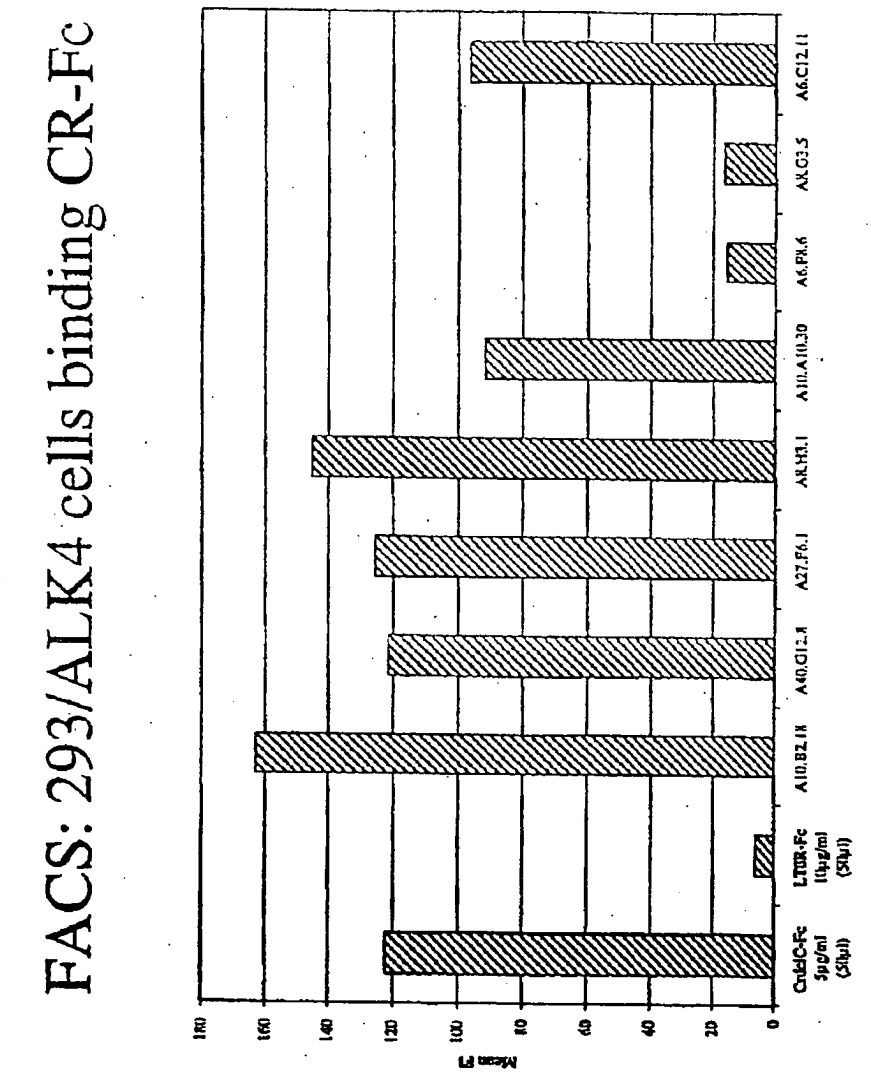


FIGURE 3



CRIPTO BLOCKING ANTIBODIES AND USES THEREOF

RELATED APPLICATIONS

This is a continuation of PCT/US02/11950, filed Apr. 17, 2002, which claims the benefit of U.S. Ser. No. 60/367,002, filed Mar. 22, 2002, and U.S. Ser. No. 60/301,091, filed Jun. 26, 2001, and U.S. Ser. No. 60/293,020, filed on May 17, 2001, and U.S. Ser. No. 60/286,782, filed on Apr. 26, 2001. The entire disclosure of each of the aforesaid patent applications are incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the fields of genetics and cellular and molecular biology. More particularly, the invention relates to antibodies which bind to and modulate the signaling of Cripto, kits comprising such antibodies, and methods which use the antibodies.

BACKGROUND OF THE INVENTION

Cripto is a cell surface protein of 188 amino acid residues serendipitously isolated in a cDNA screen of a human embryonic carcinoma library (Ciccodicola et al., 1989, EMBO J., vol. 8, no. 7, pp. 1987-1991). The Cripto protein has at least two notable domains: a cysteine-rich domain, and a domain first characterized as similar to the domain found in the epidermal growth factor (EGF) family. Cripto was originally classified as a member of the EGF family (Ciccodicola et al., supra); however, subsequent analysis showed that Cripto did not bind any of the known EGF receptors and its EGF-like domain was actually divergent from the EGF family (Bianco et al., 1999, J. Biol. Chem., 274:8624-8629).

The Cripto signaling pathway has remained elusive despite continued investigation, with the literature supporting activation of several different pathways, including a MAP kinase pathway (DeSantis et al., 1997, Cell Growth Differ., 8:1257-1266; Kannan et al., 1997, J. Biol. Chem., 272:3330-3335), the TGF-β pathway (Gritsman et al., 1999, Development, 127:921-932; Schier et al., 2000, Nature, 403:385-389), possible interactions with the Wnt pathway (Salomon et al., Endocr Relat Cancer. 2000 December;7(4):199-226; and cross talk with the EGF pathway (Bianco et al., 1999, J. Biol. Chem., 274:8624-8629).

U.S. Pat. No. 5,256,643 and two divisional applications related thereto (U.S. Pat. Nos. 5,654,140 and 5,792,616), disclose a human Cripto gene, Cripto protein, and antibodies to Cripto.

U.S. Pat. No. 5,264,557 and three divisional applications related thereto (U.S. Pat. Nos. 5,620,866, 5,650,285, and 5,854,399), disclose a human Cripto-related gene and protein. Also disclosed are antibodies which bind to the Cripto-related protein but do not cross react by binding to the Cripto protein itself.

Cripto protein overexpression is associated with many tumor types (including but not limited to breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach), as demonstrated by immunostaining of human tissue with rabbit polyclonal antibodies raised against small cripto peptides. Panico et al., 1996, Int. J. Cancer, 65: 51-56; Byrne et al., 1998, J Pathology, 185:108-111; De Angelis et al., 1999, Int J Oncology, 14:437-440. The art is therefore in need of means of controlling, restricting, and/or preventing such overexpression, modulating Cripto signaling, and

modulating the consequences of Cripto expression (i.e., promotion and/or maintenance of cell transformation).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the response of the NCCIT, human testicular carcinoma cell line, to anti-CFC blocking Cripto mAb A8G3.5.

FIG. 2 shows the response of the NCCIT, human testicular carcinoma cell line, to anti-EGF blocking Cripto mAb A27F6.1.

FIG. 3 depicts the results of the FACS analysis showing 293/ALK4 cells binding CR-Fc.

SUMMARY OF THE INVENTION

The present invention provides novel antibodies which specifically bind to Cripto, and methods of making and using such antibodies. The invention also provides antibodies which bind to Cripto, and modulate Cripto signaling or protein interaction, e.g., an antibody which binds to Cripto such that the signal resulting from a protein interaction with Cripto is modulated downward. The invention also provides antibodies which bind to Cripto and block the interaction between Cripto and ALK4. The invention also provides antibodies which bind to Cripto and modulate tumor growth. The invention also provides antibodies which bind to Cripto, modulate Cripto signaling and modulate tumor growth. The invention also provides antibodies which bind to Cripto, block the interaction between Cripto and ALK4 and modulate tumor growth

In one aspect of the invention, the antibody of the present invention specifically binds to an epitope selected from the group of epitopes to which antibodies A6C12.11, A6F8.6 (ATCC ACCESSION NO. PTA-3318), A7H1.19, A8F1.30, A8G3.5 (ATCC ACCESSION NO. PTA-3317), A8H3.1 (ATCC ACCESSION NO. PTA-3315), A8H3.2, A19A10.30, A10B2.18 (ATCC ACCESSION NO. PTA-3311), A27F6.1 (ATCC ACCESSION NO. PTA-3310), A40G12.8 (ATCC ACCESSION NO. PTA-3316), A2D3.23, A7A10.29, A9G9.9, A15C12.10, A15E4.14, A17A2.16, A17G12.1 (ATCC ACCESSION NO. PTA-3314), A17H6.1, A18B3.11 (ATCC ACCESSION NO. PTA-3312), A19E2.7, B3F6.17 (ATCC ACCESSION NO. PTA-3319), B6G7.10 (ATCC ACCESSION NO. PTA-3313), B11H8.4 bind.

In another aspect of the invention, the antibody of the present invention specifically binds to an epitope in the ligand/receptor binding domain of Cripto. Cripto can be selected from CR-1 (SEQ ID NO:1) or CR-3 (SEQ ID NO:2). In a more particular embodiment, antibodies that specifically binds to the epitope in the ligand/receptor binding domain include for example A6C12.11, A6F8.6 (ATCC ACCESSION NO. PTA-3318), A8G3.5 (ATCC ACCESSION NO. PTA-3317), A19A10.30, A8H3.1 (ATCC ACCESSION NO. PTA-3315), A27F6.1 (ATCC ACCESSION NO. PTA-3310), A40G12.8 (ATCC ACCESSION NO. PTA-3316), A17G12.1 (ATCC ACCESSION NO. PTA-3314), A18B3.11 (ATCC ACCESSION NO. PTA-3312) and B6G7.10 (ATCC ACCESSION NO. PTA-3313).

In one embodiment the epitope to which the antibodies of the present invention bind is in an EGF-like domain. Antibodies that specifically bind to the epitope in the EGF-like domain include but are not limited to A40G12.8 (ATCC ACCESSION NO. PTA-3316), A8H3.1 (ATCC ACCESSION NO. PTA-3315), A27F6.1 (ATCC ACCESSION NO. PTA-3310), B6G7.10 (ATCC ACCESSION NO. PTA-3313),

A17G12.1 (ATCC ACCESSION NO. PTA-3314) and A18B3.11 (ATCC ACCESSION NO. PTA-3312).

In another embodiment the epitope to which the antibodies of the present invention bind is in a cys-rich domain. Antibodies that specifically bind to the epitope in the cys-rich domain include but are not limited to A19A10.30, A8G3.5 (ATCC ACCESSION NO. PTA-3317), A6F8.6 (ATCC ACCESSION NO. PTA-3318) and A6C12.11.

In another embodiment the epitope to which the antibodies of the present invention bind is in the domain spanning amino acid residues 46-62 of Cripto. Antibodies that specifically bind to the epitope in the domain spanning amino acid residues 46-62 of Cripto include but are not limited to A10B2.18 (ATCC ACCESSION NO. PTA-3311), B3F6.17 (ATCC ACCESSION NO. PTA-3319) and A17A2.16.

The present inventions also comtemplate antibodies which binds specifically to Cripto and are capable of modulating Cripto signaling. Antibodies that bind specifically to Cripto and are capable of modulating Cripto signaling, include but are not limited to, A40G12.8 (ATCC ACCESSION NO. PTA-3316), A8H3.1 (ATCC ACCESSION NO. PTA-3315), A27F6.1 (ATCC ACCESSION NO. PTA-3310), and A6C12.11. In one embodiment the antibodies of the present invention which binds specifically to Cripto and are capable of modulating Cripto signaling bind to an epitope in an EGF-like domain or a cys-rich domain of Cripto.

The present inventions also comtemplate antibodies which binds specifically to Cripto and blocks the interaction between Cripto and ALK4. Antibodies that bind specifically to Cripto and are capable of blocking the interaction between Cripto and ALK4, include but are not limited to, A8G3.5 (ATCC ACCESSION NO. PTA-3317), A6F8.6 (ATCC ACCESSION NO. PTA-3318) and A6C12.11. In one embodiment the antibodies of the present invention which binds specifically to Cripto and are capable of blocking the interaction between Cripto and ALK4 bind to an epitope in an EGF-like domain or a cys-rich domain of Cripto.

In another aspect, the present invention comtemplates antibodies which bind specifically to Cripto and are capable of modulating tumor growth. Antibodies that specifically bind to Cripto and are capable of modulating tumor growth include but are not limited to, A27F6.1 (ATCC ACCESSION NO. PTA-3310), B6G7.10 (ATCC ACCESSION NO. PTA-3313) and A8G3.5 (ATCC ACCESSION NO. PTA-3317).

In one embodiment the antibodies of the present invention which bind specifically to Cripto and are capable of modulating tumor growth bind to an epitope in an EGF-like domain or a cys-rich domain of Cripto.

In yet another aspect, the present invention comtemplates antibodies which bind specifically to Cripto, which are capable of modulating Cripto signaling, and which are capable of modulating tumor growth. Antibodies that specifically bind to Cripto, which are capable of modulating Cripto signaling, and which are capable of modulating tumor growth, include but are not limited to A27F6.1 (ATCC ACCESSION NO. PTA-3310).

In one embodiment the antibodies of the present invention which bind specifically to Cripto, which are capable of modulating Cripto signaling, and which are capable of modulating tumor growth bind to an epitope in an EGF-like domain or a cys-rich domain of Cripto.

In yet another aspect, the present invention comtemplates antibodies which bind specifically to Cripto, which are capable of blocking the interaction between Cripto and ALK4, and which are capable of modulating tumor growth. Antibodies that specifically bind to Cripto, which are capable of blocking the interaction between Cripto and ALK4, and

which are capable of modulating tumor growth, include but are not limited to A8G3.5 (ATCC ACCESSION NO. PTA-3317).

The hybridoma cells A27F6.1, A10B2.18, A18B3.11, B6G7.10, A17G12.1. A8H3.1, A40G12.8, A8G3.5, A6F8.6 and B3F6.17 were deposited with the American Type Culture Collection at 12301 Parklawn Drive, Rockville, Md. 20852 under the terms of the Budapest Treaty on Jan. 19, 2001 as ATCC Accession No. PTA-3310 (A27F6.1), ATCC Accession No. PTA-3311 (A10B2.18), ATCC Accession No. PTA-3312 (A18B3.11), ATCC Accession No. PTA-3313 (B6G7.10), ATCC Accession No. PTA-3314 (A17G12.1), ATCC Accession No. PTA-3315 (A8H3.1), ATCC Accession No. PTA-3316 (A40G12.8), ATCC Accession No. PTA-3317 (A8G3.5), ATCC Accession No. PTA-3318 (A6F8.6) and ATCC Accession No. PTA-3319 (B3F6.17).

The antibodies of the present invention include but are not limited to monoclonal, polyclonal, humanized, chimeric and human antibodies.

The present invention also provides for a composition for administration to a subject having a tumor that expresses Cripto comprising at least one of the antibodies described above. In a more particular embodiment the subject is human. The composition may include a pharmaceutically acceptable excipient. The antibodies described above can be conjugated to a chemotherapeutic agent or be provided in combination with a nonconjugated chemotherapeutic.

Contemplated in another aspect of the invention are methods of modulating growth of tumor cells in vitro in a sample comprising the step of adding to the sample the compositions described above.

Also comtemplated are methods of modulating growth of tumor cells in vivo in a subject comprising the step of administering to the subject an effective amount of the compositions described above. In a particular embodiment the subject is human.

Another aspect of the invention are methods of treating subjects having a tumor that over-expresses Cripto comprising administering to the subject the compositions described above in an effective amount. Compositions for administration may include pharmaceutically acceptable excipients, antibodies conjugated to chemotherapeutic agents and antibodies administered in combination with nonconjugated chemotherapeutic agents

The methods of the present invention are particularly useful in modulating growth of tumor cells and/or treating a subject (i.e. a human) having a tumor where the tumor cell is selected from breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

In yet another embodiment, the present invention contemplates methods of determining whether a tissue expresses Cripto, comprising the step of analyzing tissue from the subject in an immunoassay using any of the antibodies described above. Also contemplated are methods of determining whether a cell line overexpresses Cripto, comprising the step of analyzing the cell line in an immunoassay using any of the antibodies described above.

These and other aspects of the invention are set forth in greater detail below in the Detailed Description of the Invention.

DETAILED DESCRIPTION OF THE INVENTION

Antibodies that specifically bind to Cripto and their uses for modulating Cripto signaling or protein interaction, and/or block the interaction between Cripto and ALK4, and/or modulate the growth of tumor cells have been discovered.

Various classes of antibodies that specifically bind to Cripto have been discovered, including, for example, antibodies that specifically bind to an epitope in the ligand/receptor binding domain of either a native Cripto protein or a denatured form of Cripto; antibodies that bind an EGF-like domain, a cys-rich domain, or a peptide (e.g., from about 3 to about 20 amino acids) from the region comprising amino acid residues 46 to 150; antibodies that bind Cripto and modulate Cripto signalling; antibodies that bind Cripto and modulate tumor cell growth; and antibodies that bind to Cripto, modulate Cripto signaling, and modulate tumor cell growth. These antibodies are selected using conventional in vitro assays for selecting antibodies which bind the ligand/receptor binding domain, modulate Cripto signaling, or modulate tumor cell growth.

The methods of this invention are useful in the therapy of malignant or benign tumors of mammals where the growth rate of the tumor (which is an abnormal rate for the normal tissue) is at least partially dependent upon Cripto. Abnormal growth rate is a rate of growth which is in excess of that required for normal homeostasis and is in excess of that for normal tissues of the same origin.

Definitions

Various definitions are made throughout this document. Most words have the meaning that would be attributed to those words by one skilled in the art. Words specifically defined either below or elsewhere in this document have the meaning provided in the context of the present invention as a whole and as are typically understood by those skilled in the art.

As used herein, the term "region" means a physically contiguous portion of the primary structure of a biomolecule. In the case of proteins, a region is defined by a contiguous portion of the amino acid sequence of that protein.

As used herein, the term "domain" refers to a structural part of a biomolecule that contributes to a known or suspected function of the biomolecule. Domains may be co-extensive with regions or portions thereof; domains may also incorporate a portion of a biomolecule that is distinct from a particular region, in addition to all or part of that region. Examples of protein domains include, but are not limited to the extracellular domain (spans from about residue 31 to about residue 188 of Cripto, including Cripto, CR-1 (SEQ ID NO: 1) and CR-3 (SEQ ID NO:2)) and transmembrane domain (spans from about residue 169 to about residue 188 of Cripto, including Cripto, CR-1 (SEQ ID NO: 1) and CR-3 (SEQ ID NO:2)). A ligand/receptor binding domain of the Cripto protein spans from about residue 75 to about residue 150 of Cripto, including Cripto, CR-1 (SEQ ID NO: 1) and CR-3 (SEQ ID NO:2) and includes the EGF-like domain of Cripto, which spans, for example, from about residue 75 to about residue 112 of Cripto, including Cripto, CR-1 (SEQ ID NO: 1) and CR-3 (SEQ ID NO:2) and the cysteine-rich domain of Cripto, which spans, for example, from about residue 114 to about residue 150 of Cripto, including Cripto, CR-1 (SEQ ID NO: 1) and CR-3 (SEQ ID NO:2). For example, many monoclonal antibodies of the present invention have been identified as binding to the EGF-like or cys-rich domains. Additionally monoclonal antibody A10B2.18 (ATCC ACCESSION NO. PTA-3311), B3F6.17 (ATCC ACCESSION NO. PTA-3319) and A17A2.16 have been identified as binding to an epitope formed in a domain in the region spanning amino acid residues 46-62, upstream of the EGF-like domain. See Example 3 below. An epitope in the ligand/receptor binding domain is an epitope, whether formed in the conformational native Cripto protein, or the denatured Cripto protein, to which antibodies may bind.

As used herein, the term "antibody" is meant to refer to complete, intact antibodies, and Fab, Fab', F(ab)2, and other fragments thereof. Complete, intact antibodies include, but are not limited to, monoclonal antibodies such as murine monoclonal antibodies, polyclonal antibodies, chimeric antibodies, human antibodies, and humanized antibodies. Various forms of antibodies may be produced using standard recombinant DNA techniques (Winter and Milstein, Nature 349: 293-99, 1991). For example, "chimeric" antibodies may be constructed, in which the antigen binding domain from an animal antibody is linked to a human constant domain (an antibody derived initially from a nonhuman mammal in which recombinant DNA technology has been used to replace all or part of the hinge and constant regions of the heavy chain and/or the constant region of the light chain, with corresponding regions from a human immunoglobulin light chain or heavy chain) (see, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. 81: 6851-55, 1984). Chimeric antibodies reduce the immunogenic responses elicited by animal antibodies when used in human clinical treatments.

In addition, recombinant "humanized" antibodies may be synthesized. Humanized antibodies are antibodies initially derived from a nonhuman mammal in which recombinant DNA technology has been used to substitute some or all of the amino acids not required for antigen binding with amino acids from corresponding regions of a human immunoglobulin light or heavy chain. That is, they are chimeras comprising mostly human immunoglobulin sequences into which the regions responsible for specific antigen-binding have been inserted (see, e.g., PCT patent application WO 94/04679). Animals are immunized with the desired antigen, the corresponding antibodies are isolated and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of the human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (inter-species) sequences in antibodies for use in human therapies, and are less likely to elicit unwanted immune responses. Primatized antibodies can be produced similarly.

Another embodiment of the invention includes the use of human antibodies, which can be produced in nonhuman animals, such as transgenic animals harboring one or more human immunoglobulin transgenes. Such animals may be used as a source for splenocytes for producing hybridomas, as is described in U.S. Pat. No. 5,569,825.

Antibody fragments and univalent antibodies may also be used in the methods and compositions of this invention. Univalent antibodies comprise a heavy chain/light chain dimer bound to the Fc (or stem) region of a second heavy chain. "Fab region" refers to those portions of the chains which are roughly equivalent, or analogous, to the sequences which comprise the Y branch portions of the heavy chain and to the light chain in its entirety, and which collectively (in aggregates) have been shown to exhibit antibody activity. A Fab protein includes aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers which correspond to the two branch segments of the antibody Y, (commonly known as F(ab)2), whether any of the above are covalently or non-covalently aggregated, so long as the aggregation is capable of specifically reacting with a particular antigen or antigen family.

Any of the antibodies of the invention may optionally be conjugated to a chemotherapeutic, as defined below.

As used herein, the term "binding" means the physical or chemical interaction between two proteins or compounds or associated proteins or compounds or combinations thereof, including the interaction between an antibody and a protein.

Binding includes ionic, non-ionic, hydrogen bonds, Van der Waals, hydrophobic interactions, etc. The physical interaction, the binding, can be either direct or indirect, indirect being through or due to the effects of another protein or compound. Direct binding refers to interactions that do not take place through or due to the effect of another protein or compound but instead are without other substantial chemical intermediates. Binding may be detected in many different manners. Methods of detecting binding are well-known to those of skill in the art.

As used herein, "an antibody capable of internalizing Cripto" means an antibody which enters the cell while removing Cripto from the cell surface. One can screen for Cripto antibodies which are capable of internalizing Cripto by using fluorescent labeled Cripto monoclonal antibodies. In order to determine which antibodies internalize into the Cripto positive cells one can assay for the uptake of the fluorescent signal of the antibodies into the cells by viewing the cells under a fluorescent and/or confocal microscope. Those antibodies that get internalized will be seen as fluorescent signals in the cytoplasmic and or cellular vesicles. Non-limiting examples of Cripto antibodies capable of internalizing Cripto include A27F6.1 and B3F6.17.

As used herein, the term "compound" means any identifiable chemical or molecule, including, but not limited to, ion, atom, small molecule, peptide, protein, sugar, nucleotide, or nucleic acid, and such compound can be natural or synthetic.

As used herein, the terms "modulates" or "modifies" means an increase or decrease in the amount, quality, or effect of a particular activity or protein.

As used herein, the term "modulate Cripto signaling" means an increase or decrease in the amount, quality, or effect of Cripto activity, by about 5%, preferably 10%, more preferably 20%, more preferably 30%, more preferably 40%, more preferably 50%, more preferably 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 100%,. Activity may be measured by assays known in the art, such as the null cell assay shown in Example 3. In another embodiment, protein interaction between Cripto and another protein is similarly modulated downward via binding of the antibodies of the invention.

As used herein, the term "blocking the interaction between Cripto and ALK 4" means an increase or decrease in the interaction, i.e. binding, between Cripto and ALK4, by about 5%, preferably 10%, more preferably 20%, more preferably 30%, more preferably 40%, more preferably 50%, more preferably 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 100%,. Activity may be measured by assays known in the art, such as the binding assay shown in Example 8.

As used herein, the term "modulate growth of tumor cells in vitro" means an increase or decrease in the number of tumor cells, in vitro, by about 5%, preferably 10%, more preferably 20%, more preferably 30%, more preferably 40%, more preferably 50%, more preferably 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 100%. In vitro modulation of tumor cell growth may be measured by assays known in the art, such as the GEO cell soft agar assay shown in Example 4.

As used herein, the term "modulate growth of tumor cells in vivo" means an increase or decrease in the number of tumor cells, in vivo, by about 5%, preferably 10%, more preferably 20%, more preferably 30%, more preferably 40%, more preferably 50%, more preferably 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 100%. In vivo modulation of tumor cell growth may be measured by assays known in the art, such as the one shown in Example 5.

The term "preventing" refers to decreasing the probability that an organism contracts or develops an abnormal condition.

The term "treating" refers to having a therapeutic effect and at least partially alleviating or abrogating an abnormal condition in the organism. Treating includes maintenance of inhibited tumor growth, and induction of remission.

The term "therapeutic effect" refers to the inhibition of an abnormal condition. A therapeutic effect relieves to some extent one or more of the symptoms of the abnormal condition. In reference to the treatment of abnormal conditions, a therapeutic effect can refer to one or more of the following: (a) an increase or decrease in the proliferation, growth, and/or differentiation of cells; (b) inhibition (i.e., slowing or stopping) or promotion of cell death; (c) inhibition of degeneration; (d) relieving to some extent one or more of the symptoms associated with the abnormal condition; and (e) enhancing the function of a population of cells. Compounds demonstrating efficacy against abnormal conditions can be identified as described herein.

The term "administering" relates to a method of incorporating a compound into cells or tissues of an organism. The abnormal condition can be prevented or treated when the cells or tissues of the organism exist within the organism or outside of the organism. Cells existing outside the organism can be maintained or grown in cell culture dishes, or in another organism. For cells harbored within the organism, many techniques exist in the art to administer compounds, including (but not limited to) oral, parenteral, dermal, injection, and aerosol applications. For cells outside of the organism, multiple techniques exist in the art to administer the compounds, including (but not limited to) cell microinjection techniques, transformation techniques and carrier techniques. Administration may be accomplished by the many modes known in the art, e.g., oral, intravenous, intraperitoneal, intramuscular, and the like. When used in in vivo therapy, the antibodies of the subject invention are administered to a patient in effective amounts. As used herein an "effective amount" is an amount sufficient to effect beneficial or desired clinical results (i.e., amounts that eliminate or reduce the patient's tumor burden). An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of the antibodies of the present invention is an amount of the antibodies that is sufficient to ameliorate, stabilize, or delay the development of the Cripto-associated disease state, particularly Cripto-associated tumors. Detection and measurement of these indicators of efficacy are discussed below. An example of a typical treatment regime includes administering by intravenous infusion to the subject antibodies of the invention on a weekly schedule, at a dose of about 2-5 mg/kg. The antibodies are administered in an outpatient chemoinfusion unit, unless the patient requires hospitalization. Other administration regimes known in the art are also contemplated.

The abnormal condition can also be prevented or treated by administering an antibody of the invention to a group of cells having an aberration in a signal transduction pathway to an organism. The effect of administering a compound on organism function can then be monitored. The organism is preferably a human.

"Cripto overexpression" is intended to mean the expression of Cripto by a tissue which expression is greater than the Cripto expression of adjacent normal tissue in a statistically significant amount.

"Chemotherapeutics" refers to any agents identified in the art as having therapeutic effect on the inhibition of tumor growth, maintenace of inhibited tumor growth, and/or induction of remission, such as natural compounds, synthetic compounds, proteins, modified proteins, and radioactive compounds. Chemotherapeutic agents contemplated herewith

include agents that can be conjugated to the antibodies of the present invention or alternatively agents that can be used in combination with the antibodies of the present invention without being conjugated to the antibody. Exemplary chemotherapeutics that can be conjugated to the antibodies of the present invention include, but are not limited to radioconjugates (90Y, 131I, 99mTc, 111In, 186Rh, et al.), tumor-activated prodrugs (maytansinoids, CC-1065 analogs, clicheamicin derivatives, anthracyclines, vinca alkaloids, et al.), ricin, diptheria toxin, pseudomonas exotoxin.

Chemotherapeutic agents may be used in combination with the antibodies of the invention, rather than being conjugated thereto (i.e. nonconjugated chemotherapeutics), include, but are not limited to the following: platinums (i.e. cis platinum), anthracyclines, nucleoside analogs (purine and pyrimidine), taxanes, camptothecins, epipodophyllotoxins, DNA alkylating agents, folate antagonists, vinca alkaloids, ribonucleotide reductase inhibitors, estrogen inhibitors, progesterone inhibitors, androgen inhibitors, aromatase inhibitors, interferons, interleukins, monoclonal antibodies, taxol, camptosar, adriamycin (dox), 5-FU and gemcitabine. Such chemotherapeutics may be employed in the practice of the invention in combination with the antibodies of the invention by coadministration of the antibody and the nonconjugated chemotherapeutic.

"Pharmaceutically acceptable carrier or excipient" refers to biologically inert compounds known in the art and employed in the administration of the antibodies of the invention. Acceptable carriers are well known in the art and are described, for example, in *Remington's Pharmaceutical Sciences*, Gennaro, ed., Mack Publishing Co., 1990. Acceptable carriers can include biocompatible, inert or bioabsorbable salts, buffering agents, oligo- or polysaccharides, polymers, viscoelastic compound such as hyaluronic acid, viscosity-improving agents, preservatives, and the like.

A "subject" refers to vertebrates, particularly members of a mammalian species, and includes but is not limited to domestic animals, sports animals, and primates, including humans.

ANTIBODIES OF THE INVENTION

The antibodies of the invention specifically bind to Cripto: As used herein, Cripto includes the CR-1 Cripto protein, the CR-3 Cripto protein, and fragments thereof. Such fragments may be entire domains, such as the extracellular or intracellular domains, the EGF-like domain, the cys-rich domain, the receptor binding domain, and the like. Such fragments may also include contiguous and noncontiguous epitopes in any domain of the Cripto protein.

In a one embodiment, the antibodies of the invention bind to an epitope in the EGF-like domain of Cripto. The EGF-like domain spans from about amino acid residue 75 to about amino acid residue 112 of the mature Cripto protein. Epitopes in the EGF-like domain may comprise linear or nonlinear spans of amino acid residues. Example of linear epitopes contemplated include but are not limited to about residues 75-85, 80-90, 85-95, 90-100, 95-105, 100-110, or 105-112. In one embodiment, the epitope in the EGF domain is an epitope formed in the conformational native Cripto protein versus a denatured Cripto protein.

In another embodiment, the antibodies of the invention bind to an epitope in the cys-rich domain of Cripto. The cys-rich domain spans from about amino acid residue 114 to about amino acid residue 150 of the mature Cripto protein. Epitopes in the cys-rich domain may comprise linear or nonlinear spans of amino acid residues. Example of linear epitopes contemplated include but are not limited to about residues 114-125, 120-130, 125-135, 130-140, 135-145, or 140-150. In one embodiment, the epitope in the cys-rich domain is an epitope formed in the conformational native Cripto protein versus a denatured Cripto protein

Once antibodies are generated, binding of the antibodies to Cripto may be assayed using standard techniques known in the art, such as ELISA, while the presence of Cripto on a cell surface may be assayed using flow cytometry (FACS), as shown in Example 2. Any other techniques of measuring such binding may alternatively be used.

The present invention provides antibodies (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, including compounds which include CDR sequences which specifically recognize a polypeptide of the invention) specific for Cripto or fragments thereof. Antibody fragments, including Fab, Fab', $F(ab')_2$, and F_v, are also provided by the invention. The terms "specific" and "selective," when used to describe binding of the antibodies of the invention, indicates that the variable regions of the antibodies of the invention recognize and bind Cripto polypeptides. It will be understood that specific antibodies of the invention may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and, in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention

The 188 amino acid sequence for CR-1 is as follows:

MDCRKMARFSYSVIWIMAISKVFELGLVAGLGHQEFARPSRGYLAFRDDS [SEQ ID NO: 1]

IWPQEEPAIRPRSSQRVPPMGIQHSKELNRTCCLNGGTCMLGSFCACPPS

FYGRNCEHDVRKENCGSVPHDTWLPKKCSLCKCWHGQLRCFPQAFLPGCD

GLVMDEHLVASRTPELPPSARTTTFMLVGICLSIQSYY

The 188 amino acid sequence for CR-3 is as follows:

MDCRKMVRFSYSVIWIMAISKAFELGLVAGLGHQEFARPSRGDLAFRDDS [SEQ ID NO: 2]

IWPQEEPAIRPRSSQRVLPMGIQHSKELNRTCCLNGGTCMLESFCACPPSF

YGRNCEHDVRKENCGSVPHDTWLPKKCSLCKCWHGQLRCFPQAFLPGCDGL

VMDEHLVASRTPELPPSARTTTFMLAGICLISIQSYY

(i.e. antibodies that specifically bind to an epitope the ligand/receptor binding domain and the domain spanning amino acid residues 46-62) are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see Harlow et al. (Eds.), *Antibodies A Laboratory Manual*; Cold Spring Harbor Laboratory; Cold Spring Harbor, N.Y. (1988), Chapter 6. Antibodies that recognize and bind fragments of Cripto protein are also contemplated, provided that the antibodies are specific for Cripto polypeptides. Antibodies of the invention can be produced using any method well known and routinely practiced in the art.

In one embodiment, the invention provides an antibody that specifically binds to an epitope in the ligand/receptor binding domain of Cripto. Antibody specificity is described in greater detail below. However, it should be emphasized that antibodies that can be generated from other polypeptides that have previously been described in the literature and that are capable of fortuitously cross-reacting with Cripto (e.g., due to the fortuitous existence of a similar epitope in both polypeptides) are considered "cross-reactive" antibodies. Such cross-reactive antibodies are not antibodies that are "specific" for Cripto. The determination of whether an antibody specifically binds to an epitope of Cripto is made using any of several assays, such as Western blotting assays, that are well known in the art. For identifying cells that express Cripto and also for modulating Cripto ligand/receptor binding activity, antibodies that specifically bind to an extracellular epitope of the Cripto protein (i.e., portions of the Cripto protein found outside the cell) are particularly useful.

In one embodiment, the invention provides a cell-free composition comprising polyclonal antibodies, wherein at least one of the antibodies is an antibody of the invention specific for Cripto. Antisera isolated from an animal is an exemplary composition, as is a composition comprising an antibody fraction of an antisera that has been resuspended in water or in another diluent, excipient, or carrier.

In another embodiment, the invention provides monoclonal antibodies. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Further in contrast to polyclonal preparations which typically include different antibodies directed against different epitopes, each monoclonal antibody is directed against a single determinant on the antigen. Monoclonal antibodies are useful to improve selectivity and specificity of diagnostic and analytical assay methods using antigen-antibody binding. Another advantage of monoclonal antibodies is that they are synthesized by a hybridoma culture, uncontaminated by other immunoglobulins. Hybridomas that produce such antibodies are also intended as aspects of the invention.

In still another related embodiment, the invention provides an anti-idiotypic antibody specific for an antibody that is specific for Cripto. For a more detailed discussion of anti-idiotypic antibodies, see, e.g., U.S. Pat. Nos. 6,063,379 and 5,780,029.

It is well known that antibodies contain relatively small antigen binding domains that can be isolated chemically or by recombinant techniques. Such domains are useful Cripto binding molecules themselves, and also may be reintroduced into human antibodies, or fused to a chemotherapeutic or polypeptide. Thus, in still another embodiment, the invention provides a polypeptide comprising a fragment of a Cripto-specific antibody, wherein the fragment and associated molecule, if any, bind to the Cripto. By way of non-limiting example, the invention provides polypeptides that are single chain antibodies and CDR-grafted antibodies. For a more detailed discussion of CDR-grafted antibodies, see, e.g., U.S. Pat. No. 5,859,205.

In another embodiment, non-human antibodies may be humanized by any of the methods known in the art. Humanized antibodies are useful for in vivo therapeutic applications. In addition, recombinant "humanized" antibodies may be synthesized. Humanized antibodies are antibodies initially derived from a nonhuman mammal in which recombinant DNA technology has been used to substitute some or all of the amino acids not required for antigen binding with amino acids from corresponding regions of a human immunoglobulin light or heavy chain. That is, they are chimeras comprising mostly human immunoglobulin sequences into which the regions responsible for specific antigen-binding have been inserted (see, e.g., PCT patent application WO 94/04679). Animals are immunized with the desired antigen, the corresponding antibodies are isolated and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of the human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (inter-species) sequences in antibodies for use in human therapies, and are less likely to elicit unwanted immune responses. Primatized antibodies can be produced similarly using primate (e.g., rhesus, baboon and chimpanzee) antibody genes. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity. See, e.g., U.S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370.

Another embodiment of the invention includes the use of human antibodies, which can be produced in nonhuman animals, such as transgenic animals harboring one or more human immunoglobulin transgenes. Such animals may be used as a source for splenocytes for producing hybridomas, as is described in U.S. Pat. No. 5,569,825, WO00076310, WO00058499 and WO00037504 and incorporated by reference herein.

Signal Modulation

In another embodiment, the antibodies of the invention bind to Cripto, and modulate Cripto signaling or Cripto-protein interactions. Over-expression of Cripto activity can lead to a de-differentiated state promoting mesenchymal cell characteristics, increased proliferation, and cell migration (Salomon et al., BioEssays 21: 61-70, 1999; Ciardiello et al., Oncogene 9: 291-298, 1994; and Baldassarre et al., Int. J. Cancer 66:538-543, 1996), phenotypes associated with cell transformation seen in neoplasia.

One method of testing the activity of anti-Cripto antibodies and their ability to modulate Cripto signaling is with an F9-Cripto knock-out (KO) cell line (Minchiotti at al., Mech. Dev. 90: 133-142, 2000). Cripto stimulates smad2 phosphorylation and the transcription factor FAST in Xenopus embryos, and the activity of the transcription factor FAST can be monitored by measuring the luciferase activity from a FAST regulatory element-luciferase reporter gene (Saijoh et al., Mol. Cell 5:35-47, 2000). F9-Cripto KO cells are deleted for the Cripto gene and are thus null for Cripto and Cripto-dependent signaling (Minchiotti at al., Mech. Dev. 90: 133-142, 2000). Cripto signaling can be assessed in the F9 Cripto KO cells by transfecting in Cripto, FAST, and the FAST regulatory element-luciferase gene construct. No Cripto dependent FAST luciferase activity will be seen in these cell lines unless Cripto cDNA, and FAST cDNA is transfected into them. Antibodies capable of blocking Cripto-dependent Nodal signaling are antibodies that block Cripto signaling function.

Other assays capable of measuring the activity of Cripto can be employed by those of skill in the art, such as a growth

in soft agar assay (see Example 4 below). The ability of cells to grow in soft agar is associated with cell transformation and the assay is a classical in vitro assay for measuring inhibition of tumor cell growth. Other assays useful in determining inhibition of activity include in vitro assays on plastic, and the like.

Therapeutic Uses

Antibodies of the invention are also useful for, therapeutic purposes, such as modulation of tumor cell growth, diagnostic purposes to detect or quantitate Cripto, and purification of Cripto.

In one embodiment of the invention, antibodies are provided which are capable of binding specifically to Cripto and which modulate growth of tumor cells in a patient. In one embodiment, the tumor cells are testicular, breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

In another embodiment, antibodies are provided which are capable of binding specifically to Cripto and which modulate growth of tumor cells which overexpress Cripto. In one embodiment, the tumor cells are cell lines which overexpress Cripto, such as cell lines derived from breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach cancer.

Anti-Cripto antibodies may be screened for in vivo activity as potential anticancer agents following standard protocols used by those of skill in the art, as illustrated in Example 4 below. Example of such protocols are outlined by the National Cancer Institute (NCI) in their "in vivo cancer models screening" protocols, NIH publication number 84-2635 (February 1984).

In another embodiment of the invention, the antibodies of the invention are used to treat a patient having a cancerous tumor.

The antibodies of the present invention can be combined with a pharmaceutically acceptable excipient and administered in a therapeutically effective dose to the patient. For a discussion of methods of inhibiting growth of tumors, see, e.g., U.S. Pat. No. 6,165,464.

Also contemplated are methods of treating a subject suffering from a disorder associated with abnormal levels (i.e. elevated or depleted) of Cripto wherein the method comprises administering to the subject an effective amount of an antibody that specifically binds to an epitope in the ligand/receptor binding domain of Cripto, including but not limited to where the epitope is in an EGF-like domain or a cys-rich domain of Cripto.

Also contemplated are methods of treating a subject suffering from a disorder associated with abnormal levels (i.e. elevated or depleted) of Cripto wherein the method comprises administering to the subject an effective amount of an antibody which specifically forms a complex with Cripto and is directed to the epitope to which an antibody selected from the group consisting of A6C12.11, A6F8.6 (ATCC ACCESSION NO. PTA-3318), A7H1.19, A8F1.30, A8G3.5 (ATCC ACCESSION NO. PTA-3317), A8H3.1 (ATCC ACCESSION NO. PTA-3315), A8H3.2, A19A10.30, A10B2.18 (ATCC ACCESSION NO. PTA-3311), A27F6.1 (ATCC ACCESSION NO. PTA-3310), A40G12.8 (ATCC ACCESSION NO. PTA-3316), A2D3.23, A7A10.29, A9G9.9, A15C12.10, A15E4.14, A17A2.16, A17C12.28, A17G12.1 (ATCC ACCESSION NO. PTA-3314), A17H6.1, A18B3.11 (ATCC ACCESSION NO. PTA-3312), A19E2.7, B3F6.17 (ATCC ACCESSION NO. PTA-3319), and B6G7.10 (ATCC ACCESSION NO. PTA-3313) is directed.

Diagnosis via detection of Cripto is readily accomplished through standard binding assays using the novel antibodies of the invention, allowing those of skill in the art to detect the presence of Cripto specifically in a wide variety of samples, cultures, and the like.

Kits comprising an antibody of the invention for any of the purposes described herein are also comprehended. In general, a kit of the invention also includes a control antigen for which the antibody is immunospecific. Embodiments include kits comprising all reagents and instructions for the use thereof.

Additional features of the invention will be apparent from the following illustrative Examples.

EXAMPLES

Example 1

Expression and Purification of Cripto

An expression plasmid designated pSGS480 was constructed by sub-cloning a cDNA encoding human Cripto amino acids residues 1 to 169 of Cripto [amino acids 1-169 of SEQ ID NO: 1], fused to human IgG_1 Fc domain (i.e., "CR (del C)-Fc") into vector pEAG1100. For a more detailed description of this vector, see copending U.S. Patent Application Ser. No. 60/233,148, filed Sep. 18, 2000. The vector pEAG1100 is a derivative of GIBCO-BRL Life Technologies plasmid pCMV-Sport-betagal, the use of which in CHO transient transfections was described by Schifferli et al., 1999, Focus 21: 16. It was made by removing the reporter gene beta-galactosidase NotI fragment from the plasmid pCMV-Sport-Betagal (catalog number 10586-014) as follows: The plasmid was digested with NotI and EcoRV, the 4.38 kb NotI vector backbone fragment was gel-purified and ligated. Ligated DNA was transformed into competent *E. coli* DH5alpha. pEAG1100 was isolated as a plasmid containing the desired recombinant from an isolated single colony. The sequence of pEAG1100 spanning the promoter, polylinker, and transcription termination signal was confirmed.

Plasmid pSGS480 was transiently transfected into CHO cells and the cells were grown at 28° C. for 7 days. The presence of CR(del C)-Fc protein in these cells and the conditioned media was examined by Western blot analysis. For Western blot analysis, conditioned media and cells from Cripto transfected cells were subjected to SDS-PAGE on 4-20% gradient gels under reducing conditions, transferred electrophoretically to nitrocellulose, and the Cripto fusion protein was detected with a rabbit polyclonal antiserum raised against a Cripto 17-mer peptide (comprising residues 97-113 of SEQ ID NO: 1)-keyhole limpet hemocyanin conjugate. After centrifugation to remove the cells, Western blot analysis showed that the CR(del C)-Fc protein was efficiently secreted into the conditioned media (supernatant). The supernatant was applied to a Protein A-Sepaharose (Pharmacia), and bound protein was eluted with 25 mM sodium phosphate pH 2.8, 100 mM NaCl. The eluted protein was neutralized with 0.5 M sodium phosphate at pH 8.6, and analyzed for total protein content from absorbance measurements at 240-340 nm, and for purity by SDS-PAGE. The eluted protein was filtered through a 0.2 micron filter, and stored at −70° C.

Example 2

Generation and Screening of Antibodies

The eluted CR(del C)-Fc protein is injected into mice, and standard hybridoma techniques known to those of skill in the art are used to generate monoclonal antibodies.

A. Generation of Antibodies

Particularly, female Robertsonian mice (Jackson Labs) were immunized intraperitoneally with 25 μg of purified human CR del C-Fc emulsified with complete fruend's adjuvant (GibcoBRL #15721-012). They were boosted two times intraperitoneally with 25 μg of CR del C-Fc emulsified with incomplete freunds's adjuvant (GibcoBRL #15720-014) and

once on Protein A beads. The sera were screened and 3 weeks after the last boost, the mouse with the best titer was boosted intraperitoneally with 50 μg soluble CR del C-Fc three days before fusion. The mouse was boosted intravenously with 50 μg CR del C-Fc the day before fusion. The mouse spleen cells were fused with FL653 myeloma cell at a 1spleen:6 myeloma ratio and were plated at 100,000, 33,000and 11,000 cells per well into 96 well tissue culture plates in selection media. Wells positive for growth were screened by FACS and ELISA a week later. Two fusions were performed.

B. Screening of Antibodies

Supernatants resulting from the first or second fusion were screened first on ELISA plates for recognition of Cripto del C and/or Cripto EGF-like domain proteins. A control fusion protein (LT-beta receptor-Fc) was coated on ELISA plates to discard monoclonal antibodies that recognized the human Fc epitope. The ELISA was performed as described below in section C. In the first fusion, primary supernatants were also screened for their ability to recognize cell surface Cripto protein on the testicular tumor cell line, NCCIT by FACS. In the case of the second fusion, the ability of supernatants to recognize Cripto on two tumor cell lines, NCCIT and the breast cancer line, DU4475 by FACs was analyzed. Secondary screens included testing the monoclonal antibody supernatant's ability to recognize cell surface Cripto on a panel of tumor cell lines (see Tables 1 and 2 for results), ability of monoclonal antibodies to recognize human Cripto immunohistochemically on human breast and colon tumor tissue sections, ability of monoclonal antibodies to block in Cripto-Nodal signalling assay, ability to block growth of tumor cell lines on plastic or in soft agar assays, and ability to internalize cell surface Cripto.

C. ELISA

The ELISA assays were performed as follows:

Materials:

Plates: Costar high-binding Easy-wash 96W plates (07-200-642)

2' antibody: Pierce Gt anti-Ms IgG (H+L)-HRP (P131430)

Substrate: Pierce TMB Substrate Kit (34021)

Stop solution: 1N H2SO4

Buffers:

Binding buffer: 0.1 M NaHPO4 pH 9.0

Blocking buffer: PBS+10% Donor Calf Serum

Wash buffer: PBS+0.1% tween-20

Antigens CR-del-C-Fc and CR-EGF-fc, control hu IgG1 fusion protein were diluted in binding buffer to 500 ng/ml. 100 μl were added per well and incubated for 1 hr at 37° C. or overnight at 4° C. The liquid was decanted and the plate inverted and blotted until dry. 250 μl/well blocking buffer was then added, followed by incubation for 30 min. at 37° C. Again, the liquid was decanted and the plate inverted and blotted until dry. Supernatants were diluted 1:50 in wash buffer, and plated at 50 μl/well, followed by incubation for 1 hour at room temperature. Plates were washed 3× vigorously with 250 μl /well wash buffer. Then 100 μl/well 2' antibody diluted in wash buffer at 1:10,000 was added, followed by incubation for 30 min. at room temperature. Plates were then washed 3× vigorously with 250 μl/well wash buffer, then substrate added at 100 μl/well. Color was permitted to develop until sufficiently dark, then 100 μl/well stop solution was added and the plates read for absorbance at 450 nm.

D. Flow Cytometry

Cripto positive cell lines may be used to assay the monoclonal antibodies for binding to Cripto using cell surface staining and flow cytometry as follows:

Release cells from T162 flasks with 2 ml PBS⁻ with 5 mM EDTA, 10 min., 37° C. Bring up to 20 ml with media with serum, pipetting up and down several times to unclump cells. Spin at 1200 rpm for 5 minutes. Wash cells with 5-10 ml 4° C. PBS with 0.1% BSA (wash buffer). Spin at 1200 rpm for 5 minutes. Resuspend at 4×10^6-10^7/ml in wash buffer. Keep on ice.

Prepare antibodies for staining. Purified antibodies are diluted to 1-10 μg/ml in wash buffer. Add 50 μl of cells to a 96-well Linbro V bottomed plate (ICN 7632105). Plate one well of cells for each control for each cell line to be analyzed, including cells for no antibody, 2° antibody only, hybridoma media, positive control antibody supernatant, if available, or purified, and an IgG subclass control (if using purified antibodies).

Plate one well of cells for each experimental sample for each cell line to be analyzed. Spin plate, 1200 rpm for 5 minutes, using a table top centrifuge at 4° C. Flick out buffer by inverting the plate and shaking until the liquid is substantially discarded. Add 40-50 μl of antibodies (or wash buffer for the no-antibody and 2° antibody-only control wells) to wells. Incubate at least 30 min.-1 hour at 4° C. Spin plate, 1200 rpm for 5 minutes. Flick out antibody solutions. Wash wells twice with 200 μl wash buffer per well, spinning after each wash. Flick out buffer.

Resuspend cells in each well in 50 μl of 1:200 dilution (in wash buffer) of R-PE tagged goat anti-mouse IgG, Fc Specific (Jackson Immunoresearch Laboratories Cat# 115-116-071). Incubate 20 min, 4° C., in the dark. Add 150 μl wash buffer to cells in each well. Spin plate at 1200 rpm for 5 minutes. Wash once with 200 μl wash buffer per well. Resuspend cells in 150 μl 1% PFA in PBS. Transfer contents of each well to separate tubes (5 ml Falcon polystyrene round bottomed tube-352052). Wrap tubes in tin foil.

The contents of the tubes are then read by flow cytometry.

The results of a two screenings of monoclonal antibodies produced by this method yielded the following results, summarized in Tables 1 and 2 below, wherein the first column provides the designated names for the hybridoma subclones, the next two columns show the results of ELISA screens, and the remaining columns show flow cytometry analysis results on four cripto-positive cell lines. The results are given in units of mean fluorescent index (MFI).

TABLE 1

Anti-Cripto Monoclonal Antibody Characterization

Hybridoma Subclone	ATCC deposit no.	ELISA Cripto delC Sups	ELISA Cripto EGFlike domain Sups	DU4475 MFI	NCCIT MFI	GEO MFI	HT3 MFI
Control-ELISA		0.06	0.07				
Control-MouseIg				14	9	37	18

TABLE 1-continued

Anti-Cripto Monoclonal Antibody Characterization

Hybridoma Subclone	ATCC deposit no.	ELISA Cripto delC Sups	ELISA Cripto EGFlike domain Sups	DU4475 MFI	NCCIT MFI	GEO MFI	HT3 MFI
A6C12.11		2.21	0.07	11	35	29	8
A6F8.6	PTA-3318	2.32	0.08	11	50	29	10
A7H1.19		2.14	0.09	14	34	27	12
A8F1.30		2.15	0.1	17	27	32	28
A8G3.5	PTA-3317	2.39	0.09	9	30	25	15
A8H3.1	PTA-3315	2.4	1.7	9	44	23	10
A8H3.2		2.54	0.07	13	13	16	14
A19A10.30		2.02	0.09	9	40	20	10
A10B2.18	PTA-3311	2.36	0.07	40	63	100	43
A27F6.1	PTA-3310	2.28	1.19	9	44	26	17
A40G12.8	PTA-3316	2.27	1.59	10	47	26	16

TABLE 2

Anti-Cripto Monoclonal Antibody Characterization

Hybridoma Subclone	ATCC deposit no.	ELISA Cripto delC	ELISA Cripto EGFlike domain	DU4475 MFI	NCCIT MFI	GEO MFI	HT3 MFI
Control-ELISA		0.05	0.05				
Control-MouseIg				10	6	4	6
A2D3.23		0.93	0.90	73	138	37	27
A7A10.29		1.37	0.07	75	83	33	83
A9G9.9		1.39	0.07	52	62	32	82
A15C12.10		1.42	0.06	46	55	25	93
A15E4.14		1.38	0.06	50	63	23	95
A17A2.16		1.40	0.06	76	97	41	81
A17C12.28		0.96	0.97	6	16	3	22
A17G12.1	PTA-3314	1.30	1.37	61	66	28	78
A17H6.1		1.38	0.05	35	30	5	28
A18B3.11	PTA-3312	1.36	1.38	50	42	33	65
A19E2.7		1.40	0.06	53	59	26	99
B3F6.17	PTA-3319	1.37	0.06	77	51	39	89
B6G7.10	PTA-3313	1.38	1.40	28	22	22	56
B11H8.4		1.41	0.06	59	101	39	107
B12C12.5		1.10	1.04	27	14	23	59
B15A2.6		1.40	0.06	36	44	22	59
C4A2.16		1.40	0.06	24	36	22	65

Example 3

Null Cell Assay for Inhibition of Cripto Signaling

The following describes an F9 Cripto null cell signaling assay used to assess inhibition of Cripto signaling.

Day 0 Coat 6 welled plates with 0.1% gelatin 2 ml/well at 37° C. for 15 min.

Seed cells at 6×10^5 F9 CRIPTO NULL cells per well.

Day 1 Transfection:

Each of the following samples is added to 300 μl Opti-Mem1 to yield Solution A for each sample:

Sample 1: 0.5 μg $(N_2)_7$ luciferase FAST reporter cDNA plus 1.5 μg empty vector cDNA.

Sample 2: 0.5 μg $(N_2)_7$ luciferase, 0.5 μg FAST, and 1 μg empty vector cDNAs.

Sample 3: 0.5 μg $(N_2)_7$ luciferase, 0.5 μg Cripto ADD 0.5 FAST, and 10.5 μg empty vector cDNAs.

Sample 4: 0.5 μg $(N_2)_7$ luciferase, 0.5 μg Cripto, 0.5 FAST, and 0.5 μg empty vector cDNAs

Sample 5: 0.5 μg $(N_2)_7$ luciferase, 0.5 μg Cripto, 0.5 FAST, and 0.5 μg empty vector cDNAs.

Sample 6: 0.5 μg $(N_2)_7$ luciferase, 0.5 μg Cripto, 0.5 FAST, and 0.5 μg empty vector cDNAs.

Sample 7: 0.5 μg $(N_2)_7$ luciferase, 0.5 μg Cripto, 0.5 FAST, and 0.5 μg empty vector cDNAs.

Sample 8: 0.5 μg $(N_2)_7$ luciferase, 0.5 μg Cripto, 0.5 FAST, and 0.5 μg empty vector cDNAs.

Sample 9: 0.5 μg $(N_2)_7$ luciferase, 0.5 μg Cripto, 0.5 FAST, and 0.5 μg empty vector cDNAs.

Solution B comprises 30 μl of Lipofectamine plus 270 μl of OptiMem1.

For each sample, mix solution A and solution B together. Incubate 45 minutes at room temperature. Rinse wells with 2 m/well of OptiMem1. Aspirate just before next step.

Add 2.4 ml of OptiMem1 to each mixture of solutions A+B, mix, add 1.5 ml/well to duplicate wells. Incubate 5 hours at 37° C. Add 1.5 ml/well of DMEM+20% FCS, 2 mM Gln, P/S to wells which received samples 1-3. Add anti-Cripto antibodies as follows: Sample 4 wells: A27F6.1, 10

µg/ml; Sample 5 wells: A27F6.1, 2 µg/ml; Sample 6 wells: A40G12.8; 10 µg/ml, Sample 7 wells: A40G12.8 2 µg/ml; Sample 8 wells: A10B2.18, 10 g/ml; Sample 9 wells: A10B2.18, 2 µg/ml.

Day 2 Remove media, wash cells with PBS, 2 ml/well. Add DMEM+0.5% FCS, 2 mM Gln, P/S with the same amounts of Cripto antibodies as the previous day, to the same wells.

Day 3 Develop luciferase signal. Wash wells with PBS+ Ca^{2+} and Mg^{2+}, 2 ml/well. Use LucLite kit, Packard cat# 6016911. Bring buffer and substrate to room temperature. Dim lights. Reconstitute substrate with 10 ml of buffer. Dilute 1:1 with PBS+Ca^{2+} and Mg^{2+}. Aspirate wells. Quickly add 250 µl of diluted substrate per well using a repeat pipettor. Swirl solution and transfer 200 µl to wells of a 96 welled white opaque bottom plate, Falcon 35-3296. Read plate on luminometer using Winglow, exporting data to Excel.

The results of this assay are summarized below in Table 3.

TABLE 3

Cripto Signaling Assay: Inhibition with Anti-Cripto Monoclonal Antibodies

cDNAs transfected	Anti-Cripto Antibody	Relative Luminescent Units
$(N_2)_7$ luc	none	123
$(N_2)_7$ luc, FAST	none	259
$(N_2)_7$ luc, FAST, Cripto	none	3091
$(N_2)_7$ luc, FAST, Cripto	A27F6.1 10 µg/ml	1507
$(N_2)_7$ luc, FAST, Cripto	A27F6.1 2 µg/ml	2297
$(N_2)_7$ luc, FAST, Cripto	A40G12.8 10 µg/ml	1213
$(N_2)_7$ luc, FAST, Cripto	A40G12.8 2 µg/ml	2626
$(N_2)_7$ luc, FAST, Cripto	A10B2.18 10 µg/ml	3466
$(N_2)_7$ luc, FAST, Cripto	A10B2.18 2 µg/ml	3103

Example 4

Assay for In Vitro Inhibition of Tumor Cell Growth

Inhibition of Cripto Signaling may also be assayed by measuring the growth of GEO cells in soft agar. See, e.g., Ciardiello et al., Oncogene. 1994 January;9(1):291-8; Ciardiello et al., Cancer Res. 1991 Feb. 1;51(3):1051-4.

First, melt 3% bactoagar. Keep at 42° C. in a water bath. Then, mix 3% bactoagar solution with prewarmed complete media to make a solution of 0.6% bactoagar, keeping at 42° C. Plate 4 mls of the solution in a 6 cm dish and let cool for at least 30 minutes to form the bottom agar layer. Trypsinize GEO cells and resuspend to 10^5 cells/ml in complete media. Add antibodies to be assayed, or controls, to the cell suspensions, titrating antibodies from 20 µg to 1 µg. Mix equal volumes of the GEO cell suspensions and 0.6% bactoagar and overlay 2 mls on top of the bottom agar layer. Let cool for at least 1 hour. Incubate for 14 days at 37° C. in CO_2 incubator. Count colonies visible without the use of a microscope. The absence of colonies, as compared to negative controls, indicates that the antibody tested inhibits in vitro tumor cell growth.

This assay was used to yield the results shown in Table 4, for the antibodies A27F6.1 and B6G7.10, both of which demonstrate the ability to decrease growth of GEO cell colonies.

TABLE 4

Results of growth in soft agar assay

Antibody	Average number of colonies
none	109.0
none	104.3
A27.F6 20 µg/ml	82.0
A27F6.1 10 µg/ml	78.3
A27F6.1 5 µg/ml	79.0
A27F6.1 1 µg/ml	108.7
B6G7.10 20 µg ml	102.3
B6G7.10 10 µg/ml	71.7

Example 5

Assay for In Vivo Inhibition of Tumor Cell Growth

To assess the inhibition of tumor cell growth, a human tumor cell line is implanted subcutaneously in athymic nude mice and the effects of the antibodies of the invention are observed, with and without additional chemotherapeutic treatments which may provide synergistic or additive effects on tumor inhibition.

This assay may be performed alternatively using different tumor cell lines, such as, for example, GEO (a well differentiated human colon cancer in-vitro cell line, is obtained from the American Tissue Type Collection (ATCC)), DU-4475 (a breast cancer in-vitro cell line obtained from the ATCC), NCCIT (a testicular tumor cell line obtained from ATCC), or others known in the art. One example of such assays is as follows:

Animals are individually marked by ear punches. The GEO cell line is passed in-vitro or in-vivo for 1-4 passages. Animals are implanted with GEO cells subcutaneously in the right flank area. The following groups of animals may be used:

Group #	Treatment	# of Mice
1.	Saline Control, 0.2 ml/mouse, i.p. three times weekly (M, W, F)	20
2.	mAb, low dose, i.p.	10
3.	mAb, middle dose, i.p.	10
4.	mAb, high dose, i.p.	10
5.	5-FU, 30 mg/kg/inj, i.p., 3 Rx/wk (M, W, F)	10
6.	Cisplatin, 2 mg/kg/inj, s.c., 3 Rx/wk (M, W, F)	10
7.	Adriamycin, 1.6 mg/kg/inj, i.p., 3 Rx/wk (M, W, F)	10
8.	Irinotecan, 10 mg/kg/inj., i.p., 5 Rx/wk (M-F)	10
9.	mAb, low dose, i.p. + 5-FU (intermediate dose)	10
10.	mAb, middle dose, i.p. + 5-FU (intermediate dose)	10
11.	mAb, high dose, i.p. + 5-FU (intermediate dose)	10
12.	mAb, low dose, i.p. + Cisplatin (intermediate dose)	10
13.	mAb, middle dose, i.p. + Cisplatin (intermediate dose)	10
14.	mAb, high dose, i.p. + Cisplatin (intermediate dose)	10
15.	mAb, low dose, i.p. + Adriamycin (intermediate dose)	10
16.	mAb, middle dose, i.p. + Adriamycin (intermediate dose)	10
17.	mAb, high dose, i.p. + Adriamycin (intermediate dose)	10
18.	mAb, low dose, i.p. + Irinotecan (intermediate dose)	10
19.	mAb, middle dose, i.p. + Irinotecan (intermediate dose)	10
20.	mAb, high dose, i.p. + Irinotecan (intermediate dose)	10

Day 0: Implant tumor, record initial body weight of animals.

Day 1: Initiate treatments as indicated above.

Day 5: Begin tumor size and body weight measurements and continue two times weekly until termination of experiment.

Initial body weight, tumor size and body weight measurements, histology at sacrifice, and immunohistochemistry analysis on tumors are examined, analyzing for Cripto expression, tumor growth, and inhibition thereof.

Example 6

In Vivo Xenograft Tumor Model—Cys-rich Blocking Anti-Cripto Antibody

To assess the response of an NCCIT, a human testicular carcinoma cell line was implanted subcutaneously with an antibody which binds to a cys-rich domain of Cripto. The experimental methods are listed below. The results are shown in FIG. 1.

Methods and Materials:	
Animals:	Athymic nude male mice were used. Animals were individually numbered by ear punches.
Tumor:	NCCIT, mediastinal mixed germ cell human testicular carcinoma in-vitro cell line originalty obtained from the American Tissue Type Collection. Cell line was passed in-vitro for six passages in RPMI-1640/10% FBS without antibiotics. Animals implanted subcutaneously with 5×10^6 cells/0.2 ml matrigel on the animals right flank.

Group #	Treatment	# of Mice
1	Vehicle Control, (25 mM sodium phosphate, 100 mM sodium chloride, pH 7.2), 0.2 ml/mouse, i.p., Q14D Treatments begin on day-1	20
2.	A8G3.5, 1 mg/kg/inj, i.p., Q14D Treatments begin on day-1	10
3.	A8G3.5, 3 mg/kg/inj, i.p., Q14D Treatments begin on day-1	10
4.	A8G3.5, 10 mg/kg/inj, i.p., Q14D Treatments begin on day-1	10
5.	Cis-platinum, 2 mg/kg/inj, s.c., 3x/wk (M, W, F) for 6 treatments	10

Treatments began on day 1

Testing schedule

Day −1:	Randomized mice into control and treatments groups. Recorded initialbody weight of animals. Administered first treatments to antibody groups. Dosing solutions were made. Treatments were blinded to the technicians until the assay was terminated.
Day 0:	Implanted tumor. Ran bacterial cultures on the tumor implanted into mice.
Day 1:	Administered first treatment to the positive chemotherapeutic group.
Day 4:	Recorded initial tumor size measurements for tumor baseline on matrigel. Continued to record tumor size and body weights on mice 2x/week. Monitored the study daily and made notations of any unusual observation on animals.
Endpoints:	Initial body weight Tumor size and body weight measurements

Example 7

In Vivo Xenograft Tumor Model—EGF-like Domain Blocking Anti-Cripto Antibody

To assess the response of an NCCIT, a human testicular carcinoma cell line was implanted subcutaneously with an antibody which binds to a EGF-like domain of Cripto. The experimental methods are listed below. The results are shown in FIG. 2.

Methods and Materials:	
Animals:	Athymic nude male mice were used. Animals were individually numbered by ear punches.
Tumor:	NCCIT, mediastinal mixed germ cell human testicular carcinoma in-vitro cell line originally obtained from the American Tissue Type Collection. Cell line was passed in-vitro for eight passages in RPMI-1640/10% FBS without antibiotics. Animals implanted subcutaneously with 5×10^5 cells/0.2 ml matrigel on the animals right flank.

Group #	Treatment	# of Mice
1.	Vehicle Control, (25 mM sodium phosphate, 100 mM sodium chloride, pH 7.2), 0.2 ml/mouse, i.p., Q14D Treatments begin on day-1	18
2.	A27F6.1, 1 mg/kg/inj, i.p., Q14D Treatments begin on day-1 with a loading dose of 2.6 mg/kg/mouse	10
3.	A27F6.1, 10 mg/kg/inj, i.p., Q14D Treatments begin on day-1 with a loading dose of 21.2 mg/kg/mouse	10
4.	Cis-platinum, 2 mg/kg/inj, s.c., 3x/wk (M, W, F) for 6 treatments	10

Treatments began on day 1.

Testing schedule

Day −1:	Randomized mice into control and treatments groups. Recorded initial body weight of animals. Administered first treatments to antibody groups. Dosing solutions were made. Treatments were blinded to the technicians until the assay was terminated.
Day 0:	Implant tumor. Ran bacterial cultures on the tumors implanted into mice. Bacterial culture were negative for contamination at 24 and 48 hours post sampling.
Day 1:	Administered first treatment to the positive chemotherapeutic group.
Day 4:	Recorded initial tumor size measurements for tumor baseline on matrigel. Continued to record tumor size and body weights on mice 2 x/week. Monitored the study daily and made notations of any unusual observation on animals.
Endpoints:	Initial body weight Tumor size and body weight measurements

Example 8

Cripto Mabs that Block ALK4 Binding

In order to assess whether Cripto-specific monoclonal antibodies can interfere with Cripto's ability to bind to Alk4, the activin type I receptor, we used flow cytometry analysis using a 293 cell line which stably expresses Alk4. To generate this cell line, 293 cells were cotransfected with a plasmid that expresses Alk4 tagged at the C-terminus with a HA epitope and a plasmid that expresses the drug, puromycin, at a 10:1 ratio. The transfected cells were then selected in puromycin until colonies formed. Colonies were then picked, expanded and then analyzed for Alk4 expression using western blotting analysis for HA. Clone 21 (293-Alk4-21) was found to express high levels of Alk4 compared to control, untransfected 293 cells.

To analyze Cripto-Alk4 binding by flow cytometry, a purified, soluble form of human Cripto (aa 1-169) fused to the Fc portion of human IgG (CrdelC-Fc) was employed. Approxi-

mately 5 μg/ml of CrdelC-Fc or control Fc protein was incubated with 3×10^5 293-Alk4-21 cells on ice for 30 minutes in 50 μl total volume of FACS buffer (PBS with 0.1% BSA). For samples containing anti-Cripto antibodies, 5 μg/ml CrdelC-Fc was preincubated with 50 μg/ml of each Cripto antibody (A10B2.18, A40.G12.8, A27.F6.1, A8.H3.1, A19.A10.30, A6.F8.6, A8.G3.5, A6.C12.11) on ice prior to addition of the cells. The cells were then washed in FACS buffer and the bound Fc protein was detected by incubating the cells with a R-phycoerytherin-conjugated goat anti-human IgG (Fc fragment specific) from Jackson Immunologics. Samples were then washed again, fixed in 1% paraformaldehyde in PBS, and analyzed using standard flow cytometry procedures. The results of the FACS assay are shown in FIG. 3.

Some of the embodiments of the invention described above are outlined below and include, but are not limited to, the following embodiments. As those skilled in the art will appreciate, numerous changes and modifications may be made to the various embodiments of the invention without departing from the spirit of the invention. It is intended that all such variations fall within the scope of the invention.

The entire disclosure of each publication cited herein is hereby incorporated by reference.

SEQUENCE LISTING

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<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

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Asp Ser Ile Trp Pro Gln Glu Glu Pro Ala Ile Arg Pro Arg Ser Ser
50 55 60

Gln Arg Val Pro Pro Met Gly Ile Gln His Ser Lys Glu Leu Asn Arg
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Thr Cys Cys Leu Asn Gly Gly Thr Cys Met Leu Gly Ser Phe Cys Ala
85 90 95

Cys Pro Pro Ser Phe Tyr Gly Arg Asn Cys Glu His Asp Val Arg Lys
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Glu Asn Cys Gly Ser Val Pro His Asp Thr Trp Leu Pro Lys Lys Cys
115 120 125

Ser Leu Cys Lys Cys Trp His Gly Gln Leu Arg Cys Phe Pro Gln Ala
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Phe Leu Pro Gly Cys Asp Gly Leu Val Met Asp Glu His Leu Val Ala
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Ser Arg Thr Pro Glu Leu Pro Pro Ser Ala Arg Thr Thr Thr Phe Met
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Leu Val Gly Ile Cys Leu Ser Ile Gln Ser Tyr Tyr
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<210> SEQ ID NO 2
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<213> ORGANISM: Homo Sapiens

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Met Ala Ile Ser Lys Ala Phe Glu Leu Gly Leu Val Ala Gly Leu Gly
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His Gln Glu Phe Ala Arg Pro Ser Arg Gly Asp Leu Ala Phe Arg Asp
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Asp Ser Ile Trp Pro Gln Glu Glu Pro Ala Ile Arg Pro Arg Ser Ser
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Gln Arg Val Leu Pro Met Gly Ile Gln His Ser Lys Glu Leu Asn Arg
65                  70                  75                  80

Thr Cys Cys Leu Asn Gly Gly Thr Cys Met Leu Glu Ser Phe Cys Ala
                85                  90                  95

Cys Pro Pro Ser Phe Tyr Gly Arg Asn Cys Glu His Asp Val Arg Lys
            100                 105                 110

Glu Asn Cys Gly Ser Val Pro His Asp Thr Trp Leu Pro Lys Lys Cys
        115                 120                 125

Ser Leu Cys Lys Cys Trp His Gly Gln Leu Arg Cys Phe Pro Gln Ala
    130                 135                 140

Phe Leu Pro Gly Cys Asp Gly Leu Val Met Asp Glu His Leu Val Ala
145                 150                 155                 160

Ser Arg Thr Pro Glu Leu Pro Pro Ser Ala Arg Thr Thr Thr Phe Met
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Leu Ala Gly Ile Cys Leu Ser Ile Gln Ser Tyr Tyr
            180                 185
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We claim:

1. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto comprised in the domain spanning amino acid residues from amino acid 46 to amino acid 62 of SEQ ID NO:1 or amino acid residues from about amino acid 46 to about amino acid 62 of SEQ ID NO:2 in an effective amount.

2. The method according to claim 1, wherein the subject is human.

3. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto comprised in the cysteine-rich domain of Cripto spanning from about amino acid residue 114 to about amino acid residue 150 of SEQ ID NO:1 or from about amino acid residue 114 to about amino acid residue 150 of SEQ ID NO:2 in an effective amount.

4. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a monoclonal antibody which binds specifically to an epitope of Cripto selected from the group of epitopes to which antibodies produced by hybridomas A6F8.6, A8G3.5, A10B2.18 and B3F6.17 bind in an effective amount.

5. The method according to claim 1, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

6. The method of claim 1, wherein the antibody is a humanized antibody.

7. The method of claim 1, wherein the antibody is a human antibody.

8. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto comprised in the domain spanning amino acid residues from amino acid 46 to amino acid 62 of SEQ ID NO:1 or from about amino acid 46 to anout amino acid 62 of SEQ ID NO:2 and a pharmaceutically acceptable carrier.

9. The method of claim 1, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

10. The method of claim 1, wherein the antibody is a full length antibody.

11. The method of claim 1, wherein the antibody is a single chain antibody.

12. The method of claim 1, wherein the antibody is conjugated to a chemotherapeutic agent.

13. The method of claim 1, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

14. The method of claim 12, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

15. The method of claim 14, wherein the agent is a maytansinoid.

16. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal that specifically binds to an epitope of Cripto comprised in the domain spanning amino acid residues from amino acid 46-62 of SEQ ID NO:1 or amino acid residues from about amino acid 46-62 of SEQ ID NO:2, wherein the antibody is conjugated to a maytansinoid, and a pharmaceutically acceptable carrier.

17. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal antibody that binds to Cripto, wherein the antibody is a humanized version of the antibody produced by the hybridoma B3F6.17.

18. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of adminis-

tering to the subject an effective amount of a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto to which an antibody produced by hybridoma B3F6.17 binds, and a pharmaceutically acceptable carrier.

19. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal antibody, wherein the antibody specifically binds to an epitope of Cripto selected from the group of epitopes to which antibodies produced by hybridomas of A27F6.1 and B3F6.17 bind, and is capable of internalizing Cripto.

20. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto comprised in the cysteine-rich domain of Cripto spanning from about amino acid residue 114 to about amino acid residue 150 of SEQ ID NO:1 or from about amino acid residue 114 to about amino acid residue 150 of SEQ ID NO:2, and a pharmaceutically acceptable carrier.

21. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal antibody that binds to Cripto, wherein the antibody specifically binds to an epitope of Cripto selected from the group of epitopes to which antibodies produced by hybridomas of A8G3.5, and A6F8.6 bind, and a pharmaceutically acceptable carrier.

22. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal antibody that specifically binds to a Cripto amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO:2 and inhibits the interaction of Cripto and ALK4.

23. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal antibody that binds specifically to an epitope of Cripto selected from the group of epitopes to which antibodies produced by hybridomas A6F8.6, A8G3.5, A10B2.18, and B3F6.17 bind, and a pharmaceutically acceptable carrier.

24. A method of inhibiting proliferation of tumor cells that express Cripto in a subject comprising the step of administering to the subject an effective amount of a composition comprising a monoclonal antibody that binds to Cripto, wherein the antibody specifically binds to an epitope of Cripto to which an antibody produced by hybridoma A10B2.18 binds, and a pharmaceutically acceptable carrier.

25. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto comprised in the domain spanning amino acid residues from amino acid 46-62 of SEQ ID NO: 1 or amino acid residues from about amino acid 46-62 of SEQ ID NO:2, wherein the antibody is conjugated to a maytansinoid, in an effective amount.

26. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a humanized version of the antibody produced by the hybridoma B3F6.17 in an effective amount.

27. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto to which an antibody produced by the hybridoma B3F6.17 binds in an effective amount.

28. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto to which an antibody produced by the hybridoma A10B2.18 binds in an effective amount.

29. A method of treating a subject having a tumor that over-expresses Cripto comprising a monoclonal antibody, wherein the antibody specifically binds to an epitope of Cripto selected from the group of epitopes to which antibodies produced by hybridomas A27F6.1 and B3F6.17 bind, and is capable of internalizing Cripto.

30. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a monoclonal antibody that specifically binds to an epitope of Cripto selected from the group of epitopes to which antibodies produced by hybridomas A8G3.5, and A6F8.6 bind in an effective amount.

31. A method of treating a subject having a tumor that over-expresses Cripto comprising administering to the subject a composition comprising a monoclonal antibody that specifically binds to a Cripto amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 2 and inhibits the interaction of Cripto and ALK4 in an effective amount.

32. The method according to claim 3, wherein the subject is human.

33. The method according to claim 3, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

34. The method of claim 3, wherein the antibody is a humanized antibody.

35. The method of claim 3, wherein the antibody is a human antibody.

36. The method of claim 3, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

37. The method of claim 3, wherein the antibody is a full length antibody.

38. The method of claim 3, wherein the antibody is a single chain antibody.

39. The method of claim 3, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

40. The method of claim 3, wherein the antibody is conjugated to a chemotherapeutic agent.

41. The method of claim 40, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

42. The antibody of claim 41, wherein the agent is a maytansinoid.

43. The method according to claim 4, wherein the subject is human.

44. The method according to claim 4, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

45. The method of claim 4, wherein the antibody is a humanized antibody.

46. The method of claim 4, wherein the antibody is a human antibody.

47. The method of claim 4, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

48. The method of claim 4, wherein the antibody is a full length antibody.

49. The method of claim 4, wherein the antibody is a single chain antibody.

50. The method of claim 4, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

51. The method of claim 4, wherein the antibody is conjugated to a chemotherapeutic agent.

52. The method of claim 51, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

53. The antibody of claim 52, wherein the agent is a maytansinoid.

54. The method according to claim 8, wherein the subject is human.

55. The method according to claim 8, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

56. The method of claim 8, wherein the antibody is a humanized antibody.

57. The method of claim 8, wherein the antibody is a human antibody.

58. The method of claim 8, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

59. The method of claim 8, wherein the antibody is a full length antibody.

60. The method of claim 8, wherein the antibody is a single chain antibody.

61. The method of claim 8, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

62. The method of claim 8, wherein the antibody is conjugated to a chemotherapeutic agent.

63. The method of claim 62, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

64. The antibody of claim 63, wherein the agent is a maytansinoid.

65. The method according to claim 16, wherein the subject is human.

66. The method according to claim 16, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

67. The method of claim 16, wherein the antibody is a humanized antibody.

68. The method of claim 16, wherein the antibody is a human antibody.

69. The method of claim 16, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

70. The method of claim 16, wherein the antibody is a full length antibody.

71. The method of claim 16, wherein the antibody is a single chain antibody.

72. The method according to claim 17, wherein the subject is human.

73. The method according to claim 17, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

74. The method of claim 17, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

75. The method of claim 17, wherein the antibody is a full length antibody.

76. The method of claim 17, wherein the antibody is a single chain antibody.

77. The method of claim 17, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

78. The method of claim 17, wherein the antibody is conjugated to a chemotherapeutic agent.

79. The method of claim 78, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

80. The antibody of claim 79, wherein the agent is a maytansinoid.

81. The method according to claim 18, wherein the subject is human.

82. The method according to claim 18, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

83. The method of claim 18, wherein the antibody is a humanized antibody.

84. The method of claim 18, wherein the antibody is a human antibody.

85. The method of claim 18, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

86. The method of claim 18, wherein the antibody is a full length antibody.

87. The method of claim 18, wherein the antibody is a single chain antibody.

88. The method of claim 18, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

89. The method of claim 18, wherein the antibody is conjugated to a chemotherapeutic agent.

90. The method of claim 89, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

91. The antibody of claim 90, wherein the agent is a maytansinoid.

92. The method according to claim 19, wherein the subject is human.

93. The method according to claim 19, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

94. The method of claim 19, wherein the antibody is a humanized antibody.

95. The method of claim 19, wherein the antibody is a human antibody.

96. The method of claim 19, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

97. The method of claim 19, wherein the antibody is a full length antibody.

98. The method of claim 19, wherein the antibody is a single chain antibody.

99. The method of claim 19, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

100. The method of claim 19, wherein the antibody is conjugated to a chemotherapeutic agent.

101. The method of claim 100, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

102. The antibody of claim 101, wherein the agent is a maytansinoid.

103. The method according to claim 20, wherein the subject is human.

104. The method according to claim 20, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

105. The method of claim 20, wherein the antibody is a humanized antibody.

106. The method of claim 20, wherein the antibody is a human antibody.

107. The method of claim 20, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

108. The method of claim 20, wherein the antibody is a full length antibody.

109. The method of claim 20, wherein the antibody is a single chain antibody.

110. The method of claim 20, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

111. The method of claim 20, wherein the antibody is conjugated to a chemotherapeutic agent.

112. The method of claim 111, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

113. The antibody of claim 112, wherein the agent is a maytansinoid.

114. The method according to claim 21, wherein the subject is human.

115. The method according to claim 21, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

116. The method of claim 21, wherein the antibody is a humanized antibody.

117. The method of claim 21, wherein the antibody is a human antibody.

118. The method of claim 21, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

119. The method of claim 21, wherein the antibody is a full length antibody.

120. The method of claim 21, wherein the antibody is a single chain antibody.

121. The method of claim 21, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

122. The method of claim 21, wherein the antibody is conjugated to a chemotherapeutic agent.

123. The method of claim 122, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

124. The antibody of claim 123, wherein the agent is a maytansinoid.

125. The method according to claim 22, wherein the subject is human.

126. The method according to claim 22, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

127. The method of claim 22, wherein the antibody is a humanized antibody.

128. The method of claim 22, wherein the antibody is a human antibody.

129. The method of claim 22, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

130. The method of claim 22, wherein the antibody is a full length antibody.

131. The method of claim 22, wherein the antibody is a single chain antibody.

132. The method of claim 22, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

133. The method of claim 22, wherein the antibody is conjugated to a chemotherapeutic agent.

134. The method of claim 133, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

135. The antibody of claim 134, wherein the agent is a maytansinoid.

136. The method according to claim 23, wherein the subject is human.

137. The method according to claim 23, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

138. The method of claim 23, wherein the antibody is a humanized antibody.

139. The method of claim 23, wherein the antibody is a human antibody.

140. The method of claim 23, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

141. The method of claim 23, wherein the antibody is a full length antibody.

142. The method of claim 23, wherein the antibody is a single chain antibody.

143. The method of claim 23, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

144. The method of claim 23, wherein the antibody is conjugated to a chemotherapeutic agent.

145. The method of claim 144, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

146. The antibody of claim 145, wherein the agent is a maytansinoid.

147. The method according to claim 24, wherein the subject is human.

148. The method according to claim 24, wherein the tumor cell is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumor cells.

149. The method of claim 24, wherein the antibody is a humanized antibody.

150. The method of claim 24, wherein the antibody is a human antibody.

151. The method of claim 24, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

152. The method of claim 24, wherein the antibody is a full length antibody.

153. The method of claim 24, wherein the antibody is a single chain antibody.

154. The method of claim 24, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

155. The method of claim 24, wherein the antibody is conjugated to a chemotherapeutic agent.

156. The method of claim 155, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

157. The antibody of claim 156, wherein the agent is a maytansinoid.

158. The method according to claim 25, wherein the subject is human.

159. The method according to claim 25, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

160. The method of claim 25, wherein the antibody is a humanized antibody.

161. The method of claim 25, wherein the antibody is a human antibody.

162. The method of claim 25, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

163. The method of claim 25, wherein the antibody is a full length antibody.

164. The method of claim 25, wherein the antibody is a single chain antibody.

165. The method according to claim 26, wherein the subject is human.

166. The method according to claim 26, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

167. The method of claim 26, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

168. The method of claim 26, wherein the antibody is a full length antibody.

169. The method of claim 26, wherein the antibody is a single chain antibody.

170. The method of claim 26, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

171. The method of claim 26, wherein the antibody is conjugated to a chemotherapeutic agent.

172. The method of claim 171, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

173. The antibody of claim 172, wherein the agent is a maytansinoid.

174. The method according to claim 27, wherein the subject is human.

175. The method according to claim 27, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

176. The method of claim 27, wherein the antibody is a humanized antibody.

177. The method of claim 27, wherein the antibody is a human antibody.

178. The method of claim 27, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

179. The method of claim 27, wherein the antibody is a full length antibody.

180. The method of claim 27, wherein the antibody is a single chain antibody.

181. The method of claim 27, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

182. The method of claim 27, wherein the antibody is conjugated to a chemotherapeutic agent.

183. The method of claim 182, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

184. The antibody of claim 183, wherein the agent is a maytansinoid.

185. The method according to claim 28, wherein the subject is human.

186. The method according to claim 28, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

187. The method of claim 28, wherein the antibody is a humanized antibody.

188. The method of claim 28, wherein the antibody is a human antibody.

189. The method of claim 28, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

190. The method of claim 28, wherein the antibody is a full length antibody.

191. The method of claim 28, wherein the antibody is a single chain antibody.

192. The method of claim 28, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

193. The method of claim 28, wherein the antibody is conjugated to a chemotherapeutic agent.

194. The method of claim 193, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

195. The antibody of claim 194, wherein the agent is a maytansinoid.

196. The method according to claim 29, wherein the subject is human.

197. The method according to claim 29, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

198. The method of claim 29, wherein the antibody is a humanized antibody.

199. The method of claim 29, wherein the antibody is a human antibody.

200. The method of claim 29, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

201. The method of claim 29, wherein the antibody is a full length antibody.

202. The method of claim 29, wherein the antibody is a single chain antibody.

203. The method of claim 29, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

204. The method of claim 29, wherein the antibody is conjugated to a chemotherapeutic agent.

205. The method of claim 204, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

206. The antibody of claim 205, wherein the agent is a maytansinoid.

207. The method according to claim 30, wherein the subject is human.

208. The method according to claim 30, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

209. The method of claim 30, wherein the antibody is a humanized antibody.

210. The method of claim 30, wherein the antibody is a human antibody.

211. The method of claim 30, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

212. The method of claim 30, wherein the antibody is a full length antibody.

213. The method of claim 30, wherein the antibody is a single chain antibody.

214. The method of claim 30, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

215. The method of claim 30, wherein the antibody is conjugated to a chemotherapeutic agent.

216. The method of claim 215, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

217. The antibody of claim 216, wherein the agent is a maytansinoid.

218. The method according to claim **31**, wherein the subject is human.

219. The method according to claim 31, wherein the tumor is selected from the group consisting of breast, testicular, colon, lung, ovary, bladder, uterine, cervical, pancreatic, and stomach tumors.

220. The method of claim 31, wherein the antibody is a humanized antibody.

221. The method of claim 31, wherein the antibody is a human antibody.

222. The method of claim 31, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, a Fab', and a F(ab')2 fragment.

223. The method of claim 31, wherein the antibody is a full length antibody.

224. The method of claim 31, wherein the antibody is a single chain antibody.

225. The method of claim 31, wherein the antibody is administered in combination with a chemotherapeutic agent which is not conjugated to the antibody.

226. The method of claim 31, wherein the antibody is conjugated to a chemotherapeutic agent.

227. The method of claim 226, wherein the chemotherapeutic agent is selected from the group consisting of a tumor-activated prodrug, a radionuclide and a toxin.

228. The antibody of claim 227, wherein the agent is a maytansinoid.

* * * * *

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,531,174 B2
APPLICATION NO. : 10/693538
DATED : May 12, 2009
INVENTOR(S) : Sanicola-Nadel et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the cover page,

[*] Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 USC 154(b) by (233) days

Delete the phrase "by 233 days" and insert -- by 210 days --

Signed and Sealed this

Twentieth Day of October, 2009

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

EXHIBIT C

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: July 10, 2009
Electronic Signature for Megan E. Williams: /Megan E. Williams/

Docket No.:BGG-A117CNRCE2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Michele Sanicola-Nadel *et al.*	Patent No.: 7,531,174
Application No.: 10/693,538	Confirmation No.: 4018
Filed: October 23, 2003	Art Unit: 1643
For: CRIPTO BLOCKING ANTIBODIES AND USES THEREOF	Examiner: Huff, Sheela J.

MS Petition
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR PATENT TERM ADJUSTMENT INCLUDING REQUEST FOR RECONSIDERATION UNDER 37 CFR §1.705(b) and (d)

Dear Sir:

1. This is a request for reconsideration of the patent term adjustment (hereinafter "PTA") of 233 days indicated on the face of the issued patent. It is respectfully requested that Patentees be granted a minimum patent term adjustment of **531 days** for the above-referenced application.

2. In compliance with 37 CFR § 1.705(d), Patentees submit herewith a "Statement Under 37 CFR § 1.702(b)(2)."

3. Patentees submit herewith a "Statement Under 37 CFR §1.702(b)(2)".

4. Patentees note that an Application for Patent Term Adjustment under 37 CFR § 1.705(b) was filed on December 19, 2008, at the time of payment of the issue fee. A Decision

on this Application for Patent Term Adjustment was issued on March 25, 2009, in which Applicants' request was held in abeyance until after issuance of the application as a patent. A copy of this Decision is submitted herewith.

5. In accordance with 37 CFR §1.705(d), Patentees hereby authorize payment the fees set forth in 37 CFR § 1.705(b)(1). Please charge the fee set forth in 37 CFR §1.18(e) ($200.00) to our Deposit Order Account No. 12-0080, under order number BGG-A117CNRCE2. Please charge any necessary additional fees or credit any overpayments to our Deposit Order Account No. 12-0080.

Dated: July 10, 2009

Respectfully submitted,

By /Megan E. Williams/
Megan E. Williams
Registration No.: 43,270
LAHIVE & COCKFIELD, LLP
1 Post Office Square
Boston, Massachusetts 02109
(617) 227-7400
(617) 742-4214 (Fax)
Attorney For Applicant

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: July 10, 2009
Electronic Signature for Megan E. Williams: /Megan E. Williams/

Docket No.: BGG-A117CNRCE2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Michele Sanicola-Nadel *et al.*	Patent No.: 7,531,174
Application No.: 10/693,538	Confirmation No.: 4018
Filed: October 23, 2003	Art Unit: 1643
For: CRIPTO BLOCKING ANTIBODIES AND USES THEREOF	Examiner: Huff, Sheela J.

MS Patent Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT UNDER 37 CFR § 1.702(b)(2)

Dear Sir:

1. This statement is respectfully submitted in support of the "Application for Patent Term Adjustment Including Request for Reconsideration Under 37 CFR §1.705(b) and (d)" for the above-referenced patent. In view of the following, it is respectfully requested that Patentees be granted a patent term adjustment of **531 days** for the above-referenced application.

2. The patent term adjustment per the "Determination of Patent Term Adjustment Under 35 U.S.C. §154(b)" as shown on the face of the issued patent is 233 days. This determination of 233 days is in error for the reasons discussed below.

3. The factual bases for the above adjustment are set forth as follows:

A. Examination Delays Pursuant to 37 CFR §1.702 and §1.703

Pursuant to 37 CFR §1.703(f), the period of adjustment of the term of the patent under §1.702 is the sum of the periods of examination delay calculated under subparagraphs (a)-(e), to the extent that such periods are not overlapping, less the sum of the periods calculated under §1.704 (the period of Applicant Delay). In the above-referenced patent, Patentees are entitled to

a period of examination delay equal to the sum of the periods of delay under §1.703(a) and (b) for the reasons set forth below.

(i) "14 Month Delay" Pursuant to §1.703(a)(1)

In accordance 37 CFR §1.703(a)(1), Patentees are entitled to a period of patent term adjustment due to the failure by the Office to mail an action under 35 U.S.C. §132 not later than 14 months after the actual filing date (*i.e.*, by December 23, 2004). As shown in the USPTO's Patent Term Adjustment Calculation Sheet (Exhibit A), the Office failed to mail an action under 35 U.S.C. §132 (a Restriction Requirement) until October 18. 2005. As such, Patentees are entitled to a period of patent term adjustment beginning December 24, 2004 and ending on October 18, 2005, the date of mailing of the Restriction Requirement by the Office. Accordingly, the period of patent term adjustment due to the 14 Month Delay by the Office is **299 days**. This is consistent with the PTA Calculation Sheet (Exhibit A) from the Office.

(ii) "Three Years Delay" Pursuant to 37 CFR §1.703(b)

Patentees respectfully submit that the Office did not comply with the requirement of 35 U.S.C. §154(b) and 37 CFR §1.702(b), which requires issuance of a patent within 3 years after the date on which the patent was filed under 35 U.S.C. §111(a). As indicated on the face of the patent, the instant patent issued on May 12, 2009. As such, there was a delay of 932 days.

However, since the exclusionary period for continued examination set forth in 37 CFR §1.702(b)(1) applies to the instant patent, the number of days in the period beginning on the date on which Patentees first filed a Request for Continued Examination (November 8, 2007) and ending on the issue date of the patent (May 12, 2009), *i.e.*, 552 days, is excluded from the period of Three Years Delay (*i.e.*, 932 days). Therefore, in accordance with 37 CFR §1.703, Patentees have calculated a maximum period of Three Years Delay based on the period of time beginning on the day after the date that is three years after the date on which the above-referenced patent was filed under 35 U.S.C. § 111(a) (*i.e.*, October 24, 2006), and ending on the date Patentees first filed a Request for Continued Examination (*i.e.*, November 8, 2007). This period of delay is **380 days.**

(iii) "4 Month PTO Issue of Patent Delay" Pursuant to 37 CFR § 1.702(a)(4)

The Office did not comply with the requirement of 37 CFR § 1.702(a)(4), which requires issuance of a patent not later than four months after the date on which the issue fee was paid under 35 USC 151 and all outstanding requirements were satisfied. As shown on the Office's PTA Calculation Sheet (Exhibit A), the Office failed to issue a patent until May 12, 2009. Therefore, Patentees are entitled to a period of patent term adjustment beginning on the day after the date that is 4 months after the date on which the issue fee was paid, *i.e.*, April 20, 2009, and ending on the date the patent issued, *i.e.*, May 12, 2009. Accordingly, the period of patent term adjustment due to the 4-Month Delay by the Office is 23 days, which is in agreement with the period calculated by the USPTO on the PTA Calculation Sheet (Exhibit A).

(iv) Calculation of Total Period of Examination Delay Pursuant to 37 CFR §1.703(f)

As set forth in 37 CFR §1.703(f), the period of examination delay based on the grounds set forth in 37 CFR §1.702 is the sum of the period of 14 Month Delay (299 days), the minimum period of Three Years Delay (380 days), and the 4 Month Issue of Patent Delay (23 days), to the extent these periods of delay are not overlapping. As the period of 14 Month Delay ended on October 18, 2005, prior to the first day of the period of Three Years Delay, *i.e.*, October 24, 2006, and the period of Three Years Delay ended on November 8, 2007, prior to the first day of the period of 4 Month Issue of Patent Delay, *i.e.*, April 20, 2009, Patentees submit that these periods are not overlapping. Accordingly, the sum of the total examination delays results in a total period of examination delay of 702 days.

B. "Applicant Delay" Pursuant to 37 CFR §1.704

Pursuant to 37 CFR §1.704 the period of adjustment of the term of the patent due to examination delay is reduced by the period of Applicant Delay. As shown in the USPTO's PTA Calculation Sheet (Exhibit A), the Office has calculated a period of Applicant Delay of 171 days.

(i) Applicant Delay for Filing an Information Disclosure Statement

Patentees respectfully submit that a period of Applicant Delay of **19 days** accrued for the delayed submission of an Information Disclosure Statement (IDS) on February 6, 2006. This IDS was filed without a statement under 37 CFR §1.704(d), 19 days after the filing of a response by Patentees to the Restriction Requirement on January 18, 2006. This period is consistent with the PTA Calculation Sheet (Exhibit A) from the Office. Pursuant to 37 CFR §1.704(c)(8), this 19 day period should be considered an Applicant Delay and should be added to the period of delay by Applicants.

(ii) Applicant Delay for Responding to Non-Final Action Dated March 16, 2006

Patentees respectfully submit that an additional period of Applicant Delay of 61 days accrued for the delayed submission of an Amendment and Response on August 16, 2006. This is consistent with the PTA Calculation Sheet (Exhibit A) from the Office. Accordingly, pursuant to 37 CFR §1.704(b), this 61 day period should be considered an Applicant Delay and should be added to the period of delay by Applicants.

(iii) Applicant Delay for Responding to Non-Final Action Dated November 1, 2006

Patentees respectfully submit that an additional period of Applicant Delay of 89 days accrued for the delayed filing of a Response to Non-Final Action on May 1, 2007. This is consistent with the PTA Calculation Sheet (Exhibit A) from the Office. Accordingly, pursuant to 37 CFR §1.704(b), this 89 day period should be considered an Applicant Delay and should be added to the period of delay by Applicants.

(iv) Applicant Delay for Responding to Notice of Allowance Dated February 25, 2008

Patentees respectfully submit that an additional period of Applicant Delay of **2 days** accrued for the delayed filing of a Request for Continued Examination and Information Disclosure Statement on May 27, 2008. This is consistent with the PTA Calculation Sheet (Exhibit A) from the Office. Accordingly, pursuant to 37 CFR §1.704(b), this 2 day period should be considered an Applicant Delay and should be added to the period of delay by Applicants.

(iv) Calculation of the Total Period of Applicant Delay

In view of the above, Patentees have calculated a total period of Applicant Delay of **171 days**, which is the sum of the following Applicant Delays: (i) the 19 day period ; (ii) the 61 day period; (iii) the 89 day period ; and (iv) the 2 day period. Accordingly, Patentees respectfully submit that the period of Applicant Delay is 171 days. This is consistent with the PTA Calculation Sheet (Exhibit A) from the Office.

C. Calculation of Correct Patent Term Adjustment Pursuant to 37 CFR §1.702(f)

As set forth in 37 CFR §1.703(f), Patentees are entitled to a period of patent term adjustment equal to the period of Examination Delay reduced by the period of Applicant Delay. Therefore, Patentees submit that the correct patent term adjustment for the above-referenced application is **531 days**, which is the difference between the total period of examination delay (702 days) and the period of Applicant Delay (171 days).

4. In accordance with 37 CFR § 1.705(b)(2)(iii), Patentees submit that this patent is not subject to a terminal disclaimer.

In view of the foregoing, it is respectfully requested that the accompanying Application for Patent Term Adjustment be favorably considered and that a corrected Determination of Patent Term Adjustment be issued to reflect a patent term adjustment of **531 days**.

Dated: July 10, 2009

Respectfully submitted,

By /Megan E. Williams/
Megan E. Williams
Registration No.: 43,270
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28 State Street
Boston, Massachusetts 02109
(617) 227-7400
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Attorney For Applicant

10/693,538	CRIPTO BLOCKING ANTIBODIES AND USES THEREOF	07-10-2009::17:46:09

Patent Term Adjustments

Patent Term Adjustment (PTA) for Application Number: 10/693,538

Filing or 371(c) Date:	10-23-2003	USPTO Delay (PTO) Delay (days):	404
Issue Date of Patent:	05-12-2009	Three Years:	-
Pre-Issue Petitions (days):	+0	Applicant Delay (APPL) Delay (days):	171
Post-Issue Petitions (days):	+0	Total PTA (days):	233
USPTO Adjustment(days):	+0	Explanation Of Calculations	

Patent Term Adjustment History

Date	Contents Description	PTO(Days)	APPL(Days)
04-22-2009	PTA 36 Months	82	
05-12-2009	Patent Issue Date Used in PTA Calculation	23	
03-30-2009	Dispatch to FDC	↑	
03-25-2009	Mail-Petition Decision - Dismissed	↑	
03-25-2009	Petition Decision - Dismissed	↑	
12-19-2008	Petition Entered	↑	
12-23-2008	Application Is Considered Ready for Issue	↑	
12-19-2008	Issue Fee Payment Verified	↑	
12-19-2008	Issue Fee Payment Received		
12-12-2008	Sequence Forwarded to Pubs on Tape		
11-26-2008	Mail Notice of Allowance		
11-24-2008	Document Verification		
11-24-2008	Examiner's Amendment Communication		
11-24-2008	Notice of Allowance Data Verification Completed		
10-01-2008	Date Forwarded to Examiner		
08-26-2008	Response after Non-Final Action		
06-16-2008	Mail Non-Final Rejection		
06-11-2008	Non-Final Rejection		
05-27-2008	Information Disclosure Statement considered		
06-10-2008	Case Docketed to Examiner in GAU		
05-27-2008	Reference capture on IDS		
05-27-2008	Information Disclosure Statement (IDS) Filed		
06-04-2008	Date Forwarded to Examiner		
05-27-2008	Request for Continued Examination (RCE)		2
06-04-2008	DISPOSAL FOR A RCE/CPA/129 (express abandonment if CPA)		↑
06-04-2008	Case Docketed to Examiner in GAU		↑
05-27-2008	Information Disclosure Statement (IDS) Filed		↑
05-27-2008	Workflow - Request for RCE - Begin		↑
03-10-2008	Sequence Forwarded to Pubs on Tape		↑
02-25-2008	Mail Notice of Allowance		↑
02-25-2008	Mail Examiner's Amendment		
02-21-2008	Notice of Allowance Data Verification Completed		
01-31-2008	Document Verification		

01-18-2008	Examiner's Amendment Communication		
11-08-2007	Information Disclosure Statement considered		
11-19-2007	Date Forwarded to Examiner		
11-08-2007	Request for Continued Examination (RCE)		
11-19-2007	DISPOSAL FOR A RCE/CPA/129 (express abandonment if CPA)		
11-08-2007	Miscellaneous Incoming Letter		
11-08-2007	Reference capture on IDS		
11-08-2007	Information Disclosure Statement (IDS) Filed		
11-08-2007	Information Disclosure Statement (IDS) Filed		
11-08-2007	Workflow - Request for RCE - Begin		
11-13-2007	Correspondence Address Change		
09-05-2007	Correspondence Address Change		
08-17-2007	Sequence Forwarded to Pubs on Tape		
08-09-2007	Mail Notice of Allowance		
08-09-2007	Mail Examiner's Amendment		
08-06-2007	Notice of Allowance Data Verification Completed		
08-06-2007	Case Docketed to Examiner in GAU		
07-23-2007	Document Verification		
07-19-2007	Examiner's Amendment Communication		
05-16-2007	Date Forwarded to Examiner		
05-01-2007	Response after Non-Final Action		89
05-01-2007	Request for Extension of Time - Granted		
11-01-2006	Mail Non-Final Rejection		
10-30-2006	Non-Final Rejection		
07-27-2006	Information Disclosure Statement considered		
08-30-2006	Date Forwarded to Examiner		
08-16-2006	Response after Non-Final Action		61
08-16-2006	Request for Extension of Time - Granted		
08-16-2006	Affidavit(s) (Rule 131 or 132) or Exhibit(s) Received		
07-27-2006	Reference capture on IDS		
07-27-2006	Information Disclosure Statement (IDS) Filed		
07-27-2006	Information Disclosure Statement (IDS) Filed		
03-16-2006	Mail Non-Final Rejection		
03-06-2006	Non-Final Rejection		
02-06-2006	Information Disclosure Statement considered		
02-06-2006	Reference capture on IDS		
02-06-2006	Information Disclosure Statement (IDS) Filed		19
02-06-2006	Information Disclosure Statement (IDS) Filed		
01-31-2006	Date Forwarded to Examiner		
01-18-2006	Response to Election / Restriction Filed		
01-18-2006	Request for Extension of Time - Granted		
10-18-2005	Mail Restriction Requirement	299	
10-17-2005	Requirement for Restriction / Election		
06-28-2005	Case Docketed to Examiner in GAU		

04-11-2005	Case Docketed to Examiner in GAU	
12-01-2004	IFW TSS Processing by Tech Center Complete	
12-01-2004	Case Docketed to Examiner in GAU	
04-23-2004	Application Return from OIPE	
04-23-2004	Application Return TO OIPE	
04-22-2004	Application Dispatched from OIPE	
04-23-2004	Application Is Now Complete	
04-07-2004	Additional Application Filing Fees	
10-23-2003	Claim Preliminary Amendment	
04-07-2004	A statement by one or more inventors satisfying the requirement under 35 USC 115, Oath of the Applic	
03-12-2004	Notice Mailed--Application Incomplete--Filing Date Assigned	
01-20-2004	Cleared by L&R (LARS)	
01-20-2004	Cleared by L&R (LARS)	
01-15-2004	Referred to Level 2 (LARS) by OIPE CSR	
12-12-2003	IFW Scan & PACR Auto Security Review	
11-12-2003	CRF Is Good Technically / Entered into Database	
10-23-2003	CRF Disk Has Been Received by Preexam / Group / PCT	
10-23-2003	Initial Exam Team nn	

Close Window

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FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON MA 02109-2127

MAILED

MAR 25 2009

OFFICE OF PETITIONS

In re Application of :
SANICOLA-NADEL et al. :
Application No. 10/693,538 : DECISION ON APPLICATION
Filed: 10/23/2003 : FOR
Atty. Docket No BGN-A117CNRCE2 : PATENT TERM ADJUSTMENT

This is a decision on the APPLICATION FOR PATENT TERM ADJUSTMENT INCLUDING REQUEST FOR RECONSIDERATION UNDER 37 CFR §1.705(b) filed December 19, 2008. Applicant requests correction of the patent term adjustment from 128 days to 508 days on the basis that the Office will take in excess of three years to issue this patent.

As the instant application for patent term adjustment requests reconsideration of the patent term adjustment as it relates to the Office's failure to issue the patent within three years of the filing date, a decision is being held in abeyance until after the actual patent date. Knowledge of the actual date the patent issues is required to calculate the amount, if any, of additional patent term patentee is entitled to for Office failure to issue the patent within three years. See 37 CFR 1.703(b). (This is true even in this instance where a request for continued examination (RCE) was filed. The computer will not undertake the § 1.703(b) calculation until the actual date of issuance of the patent has been determined. Accordingly, it is still too soon to make a determination as to the correctness of any period of adjustment that will or will not be entered pursuant to § 1.703(b)).

Applicant is given TWO (2) MONTHS from the issue date of the patent to file a written request for reconsideration of the patent term adjustment for Office failure to issue the patent within three years. A copy of this decision should accompany the request. Applicant may seek such consideration without payment of an additional fee. However, as to all other bases for seeking reconsideration of the patent term adjustment indicated in the patent, all requirements of § 1.705(d) must be met. Requests for reconsideration on other bases must be timely filed and must include payment of the required fee.

Rather than file the request for reconsideration of Patent Term Adjustment at the time of the mailing of the notice of allowance, applicant is advised that they may wait until the time of the issuance of the patent and file a request for reconsideration of the patent term pursuant to 37 CFR 1.705(d). The USPTO notes that it does not calculate the amount of time earned pursuant to 37 CFR 1.702(b) until the time of the issuance of the patent and accordingly, the Office will consider any request for reconsideration of the patent term adjustment due to an error in the calculation of 37 CFR 1.702(b) to be timely if the request for reconsideration is filed within two months of the issuance of the patent.

It is acknowledged that applicant is correct that any period of adjustment will be entered in light of 35 U.S.C. 154(B) GUARANTEE OF NO MORE THAN 3-YEAR APPLICATION PENDENCY, which provides that:

> Subject to the limitations under paragraph (2), if the issue of an original patent is delayed due to the failure of the United States Patent and Trademark Office to issue a patent within 3 years after the actual filing date of the application in the United States, not including —
>
> (i) any time consumed by continued examination of the application requested by the applicant under section 132(b);

It is noted that a Request for Continued Examination (RCE) was first filed in this application on November 8, 2007.

The Office acknowledges submission of the $200.00 fee set forth in 37 CFR 1.18(e). No additional fees are required.

The application is being forwarded to the Office of Data Management for issuance of the patent. The patent term adjustment indicated on the patent (as shown on the Issue Notification mailed about three weeks prior to patent issuance) will include any additional adjustment accrued both for Office delay in issuing the patent more than four months after payment of the issue fee and satisfaction of all outstanding requirements, and for the Office taking in excess of three years to issue the patent (to the extent that the three-year period does not overlap with periods already accorded).

Telephone inquiries specific to this decision should be directed to Senior Petitions Attorney, Christina Tartera Donnell, at (571) 272-3211.

Nancy Johnson
Senior Petitions Attorney
Office of Petitions

EXHIBIT D

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
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BOSTON MA 02109-2127

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JUL 27 2009

OFFICE OF PETITIONS

In re Patent No. 7,531,174 :
Sanicola-Nadel et al. : DECISION ON REQUEST FOR
Issue Date: May 12, 2009 : RECONSIDERATION OF
Application No. 10/693,538 : PATENT TERM ADJUSTMENT
Filed: October 23, 2003 : AND NOTICE OF INTENT TO
Docket No. BGG-A117CNRCE2 : ISSUE A CERTIFICATE OF CORRECTION

This is in response to the APPLICATION FOR PATENT TERM ADJUSTMENT INCLUDING REQUEST FOR RECONSIDERATION UNDER 37 CFR §1.705(b) and (d), filed July 10, 2009, which is being treated as a petition under 37 CFR 1.705(d). Patentees request that the determination of patent term adjustment be corrected from two hundred thirty-three (233) days to five hundred thirty-one (531) days.

The request for reconsideration of the patent term adjustment indicated in the patent is **GRANTED** to the extent indicated herein.

Patentees are given **THIRTY (30) DAYS or ONE (1) MONTH, whichever is longer**, from the mail date of this decision to respond. No extensions of time will be granted under 37 CFR 1.136.

The patent term adjustment indicated on the patent is to be corrected by issuance of a certificate of correction showing a revised Patent Term Adjustment of **210 days**.

BACKGROUND

On May 12, 2009, the application matured into U.S. Patent No. 7,531,174, with a revised patent term adjustment of 233 days.

On July 10, 2009, patentees timely submitted this request for reconsideration of patent term adjustment within two months of the issue date of the patent. See 37 CFR 1.705(d).

Patentees request recalculation of the patent term adjustment. Patentees assert, pursuant to 37 CFR 1.703(f), the period of adjustment of the term of the patent under 37 CFR 1.702 is the sum of the periods of examination delay calculated under subparagraphs (a)-(e), to the extent that such periods are not overlapping, less the sum of the periods calculated under 37 CFR 1.704 (the period of applicant delay). Patentees contend no periods of delay attributable to grounds specified under 35 U.S.C. 154(b)(1)(A) and 35 U.S.C. 154(b)(1)(B) overlap. Therefore, patentees maintain that they are entitled to the sum of 322 (299 + 23) days of examination delay plus 380 days of Three Year Delay, reduced by 171 (19 + 61 + 89 + 2) days of applicant delay, for a total patent term adjustment of 531 days.

OPINION

At the outset, the Office notes that the correct period of adjustment under 37 CFR 1.702(b) for failure by the Office to issue the patent within three years is 381 days, not 380 days as stated by patentees. Furthermore, it appears that patentees used the incorrect number of 380 days in calculating the requested patent term determination of 531 (322 + 380 - 171) days.

Pursuant to 37 CFR 1.703(b), the period of adjustment of 381 day is calculated as the number of days in the period beginning on the day after the date that is three years after the date on which the application was filed under 35 U.S.C. 111(a), October 24, 2006, and ending on the date the RCE was filed November 8, 2007.[1] "When a period is indicated (in 37 CFR 1.703 or 1.704) as

---

[1] Pursuant to 35 U.S.C. 154(b)(1)(B), 37 CFR 1.702(b) provides, in pertinent part, that:

> Failure to issue a patent within three years of the actual filing date of the application. Subject to the provisions of 35 U.S.C. 154(b) and this subpart, the term of an original patent shall be adjusted if the issuance of the patent was delayed due to the failure of the Office to issue a patent within three years after the date on which the application was filed under 35 U.S.C. 111(a) or the national stage commenced under 35 U.S.C. 371(b) or (f) in an international application, but not including:

'beginning' on a particular day, that day is included in the period, in that such day is 'day one' of the period and not 'day zero.'" MPEP 2731. "For example, a period beginning on April 1 and ending on April 10 is ten (and not nine) days in length." Id.

As to patentees' interpretation of the period of overlap, the Office finds that it is inconsistent with the Office's interpretation of the overlap provision, 35 U.S.C. 154(b)(2)(A). 35 U.S.C. 154(b)(2)(A) limits the adjustment of patent term, as follows:

> to the extent that the periods of delay attributable to grounds specified in paragraph (1) overlap, the period of any adjustment granted under this subsection shall not exceed the actual number of days the issuance of the patent was delayed.

As explained in *Explanation of 37 CFR 1.703(f)*[2] *and of the United States Patent and Trademark Office Interpretation of 35 U.S.C. 154(b)(2)(A)*, 69 Fed. Reg. 34283 (June 21, 2004), the Office interprets 35 U.S.C. 154(b)(2)(A) as permitting either patent term adjustment under 35 U.S.C. 154(b)(1)(A)(i)-(iv), or patent term adjustment under 35 U.S.C. 154(b)(1)(B), but not as permitting patent term adjustment under both 35 U.S.C. 154(b)(1)(A)(i)-(iv) and 154(b)(1)(B). Accordingly, the Office implements the overlap provision as follows:

> If an application is entitled to an adjustment under 35 U.S.C. 154(b)(1)(B), the entire period during which the application was pending (except for periods excluded under 35 U.S.C. 154(b)(1)(B)(i)-(iii)), and not just the period beginning three years after the actual filing date of the application, is the period of delay under 35 U.S.C. 154(b)(1)(B) in determining whether periods of delay

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(i) any time consumed by continued examination of the application requested by the applicant under section 132(b).

[2] Likewise, 37 CFR 1.703(f) provides that:

> To the extent that periods of delay attributable to the grounds specified in § 1.702 overlap, the period of adjustment granted under this section shall not exceed the actual number of days the issuance of the patent was delayed.

> overlap under 35 U.S.C. 154(b)(2)(A). Thus, any days of delay for Office issuance of the patent more than 3 years after the filing date of the application, which overlap with the days of patent term adjustment accorded prior to the issuance of the patent will not result in any additional patent term adjustment. See 35 U.S.C. 154(b)(1)(B), 35 U.S.C. 154(b)(2)(A), and 37 CFR § 1.703(f). See *Changes to Implement Patent Term Adjustment Under Twenty Year Term; Final Rule*, 65 Fed. Reg. 54366 (Sept. 18, 2000). See also *Revision of Patent Term Extension and Patent Term Adjustment Provisions; Final Rule*, 69 Fed. Reg. 21704 (April 22, 2004), 1282 Off. Gaz. Pat. Office 100 (May 18, 2004). See also *Explanation of 37 CFR 1.703(f) and of the United States Patent and Trademark Office Interpretation of 35 U.S.C. 154(b)(2)(A)*, 69 Fed. Reg. 34283 (June 21, 2004).

Further, as stated in the *Explanation of 37 CFR 1.703(f) and of the United States Patent and Trademark Office Interpretation of 35 U.S.C. 154(b)(2)(A)*, the Office has consistently taken the position that if an application is entitled to an adjustment under the three-year pendency provision of 35 U.S.C. 154(b)(1)(B), the entire period during which the application was pending before the Office (except for periods excluded under 35 U.S.C. 154(b)(1)(B)(i)-(iii)), and not just the period beginning three years after the actual filing date of the application, is the relevant period under 35 U.S.C. 154(b)(1)(B) in determining whether periods of delay "overlap" under 35 U.S.C. 154(b)(2)(A).

This interpretation is consistent with the statute. Taken together the statute and rule provide that to the extent that periods of delay attributable to grounds specified in 35 U.S.C. 154(b)(1) and in corresponding 37 CFR 1.702 overlap, the period of adjustment granted shall not exceed the actual number of days the issuance of the patent was delayed.

It is noted, however, that delays resulting in the Office's failure to meet the time frames specified in 35 U.S.C. 154(b)(1)(A) (the "fourteen-four-four-four" provisions) are not always overlapping with a delay resulting in the Office's failure to issue a patent within the three-year time frame specified in 35 U.S.C. 154(b)(1)(B) because not all application pendency time is counted toward this three-year period. See 35 U.S.C. 154(b)(1)(B)(i)-(iii).

In this instance, all application pendency time is not counted toward the three-year period. A request for continued examination was filed on November 8, 2007. The period subsequent to the filing of the RCE is not included in the three-year time frame specified in 35 U.S.C. 154(b)(1)(B). See 35 U.S.C. 154(b)(1)(B)(i). Thus, the relevant period under 35 U.S.C. 154(b)(1)(B) in determining whether periods of delay "overlap" under 35 U.S.C. 154(b)(2)(A) is the period from October 23, 2003 to November 8, 2007. Thus, only the 299 days[3] of patent term adjustment accorded prior to the filing of the RCE pursuant to 37 CFR 1.702(a)(1)[4] are considered in determining overlap. The 23 days[5] for Office delay under 37 CFR 1.702(a)(4),[6] occurring subsequent to the filing of the RCE are not considered. The 381 days attributed to Office delay pursuant to 37 CFR 1.702(b) is determined to overlap with the

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[3] The Office mailed a Restriction Requirement on October 18, 2005, 14 months and 299 days after the filing of the application on October 23, 2003.

[4] 37 CFR 1.702, provides grounds for adjustment of patent term due to examination delay under the Patent Term Guarantee Act of 1999 (original applications, other than designs, filed on or after May 29, 2000).

> (a) Failure to take certain actions within specified time frames. Subject to the provisions of 35 U.S.C. 154(b) and this subpart, the term of an original patent shall be adjusted if the issuance of the patent was delayed due to the failure of the Office to:
>
> (1) Mail at least one of a notification under 35 U.S.C. 132 or a notice of allowance under 35 U.S.C. 151 not later than fourteen months after the date on which the application was filed under 35 U.S.C. 111(a) or fulfilled the requirements of 35 U.S.C. 371 in an international application[.]

[5] The Office issued the patent on May 12, 2009, 4 months and 23 days after the payment of the issue fee on December 19, 2008.

[6] 37 CFR 1.702, provides grounds for adjustment of patent term due to examination delay under the Patent Term Guarantee Act of 1999 (original applications, other than designs, filed on or after May 29, 2000).

> **(a) Failure to take certain actions within specified time frames. Subject to the provisions of 35 U.S.C. 154(b) and this subpart, the term of an original patent shall be adjusted if the issuance of the patent was delayed due to the failure of the Office to:**
>
> **. . .**
>
> **(4) Issue a patent not later than four months after the date on which the issue fee was paid under 35 U.S.C. 151 and all outstanding requirements were satisfied.**

299 days attributed to Office delay pursuant to 1.702(a)(1). 381 days is the actual number of days issuance of the patent was delayed. Accordingly, at issuance, the Office should have entered only 59 additional days of patent term adjustment (not 82 days) for the Office taking in excess of three year to issue the patent for a total Office delay of 381 (299 + 23 + 59) days. Accordingly, the additional 82 days will be removed and 59 days will be entered.

In view thereof, the revised determination of patent term adjustment at the time of the issuance of the patent is 210 days (381 days of Office delay - 171 days of applicant delay).

The Office acknowledges the $200.00 fee under 37 CFR 1.18(e) paid on December 9, 2008, and July 10, 2009. The decision of March 25, 2009, stated that an additional petition fee for seeking reconsideration of patent term adjustment as it relates to the Three Year Delay was not necessary if filed within two month of the issuance of the patent. Therefore, the $200.00 fee paid on July 10, 2009, will be refunded. No additional fees are required.

The application file is being forwarded to the Certificates of Correction Branch for issuance of a certificate of correction in order to rectify this error. The Office will issue a certificate of correction indicating that the term of the above-identified patent is extended or adjusted by 210 days.

Telephone inquiries regarding this matter should be directed to the undersigned at (571) 272-3211.

Christina Tartera Donnell

Christina Tartera Donnell
Senior Petitions Attorney
Office of Petitions

Enclosure: Copy of DRAFT Certificate of Correction

DRAFT COPY

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT : 7,531,174 B2

DATED : May 12, 2009

INVENTOR(S) : Sanicola-Nadel et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the cover page,

[*] Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 USC 154(b) by (233) days

Delete the phrase "by 233 days" and insert – by 210 days--

EXHIBIT E

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

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FLOOR 30, SUITE 3000
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BOSTON MA 02109-2127

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SEP 0 1 2009

OFFICE OF PETITIONS

In re Patent No. 7,531,174	:
Sanicola-Nadel et al.	: DECISION ON REQUEST FOR
Issue Date: May 12, 2009	: RECONSIDERATION OF
Application No. 10/693,538	: PATENT TERM ADJUSTMENT
Filed: October 23, 2003	: AND NOTICE OF INTENT TO
Docket No. BGG-A117CNRCE2	: ISSUE A CERTIFICATE OF CORRECTION

This is in response to the RESPONSE TO DECISION ON REQUEST FOR RECONSIDERATION OF PATENT TERM ADJUSTMENT AND NOTICE OF INTENT TO ISSUE CERTIFICATE OF CORRECTION, filed August 26, 2009, which is properly treated as a petition under 37 CFR 1.705(d). Patentees request that the determination of patent term adjustment be corrected from two hundred ten (210) days to five hundred thirty-one (531) days, or in the alternative, two hundred thirty-two (232) days.

The request for reconsideration of the patent term adjustment indicated in the patent is GRANTED to the extent indicated herein.

Patentees are given **THIRTY (30) DAYS or ONE (1) MONTH, whichever is longer**, from the mail date of this decision to respond. No extensions of time will be granted under 37 CFR 1.136.

The patent term adjustment indicated on the patent is to be corrected by issuance of a certificate of correction showing a revised Patent Term Adjustment of two hundred thirty-three (233) days.

BACKGROUND

On May 12, 2009, the application matured into U.S. Patent No. 7,531,174, with a revised patent term adjustment of 233 days

(404 days of Office delay - 171 days of applicant delay). On July 10, 2009, patentees timely submitted a request for reconsideration of patent term adjustment within two months of the issue date of the patent. On July 27, 2009, the Office mailed a decision on the request stating that the patent term adjustment indicated on the patent would be corrected by the issuance of a certificate of correction showing a revised patent term adjustment of 210 days. The Office issued the certificate of correction on August 25, 2009. On August 26, 2009, patentees mailed a "RESPONSE TO DECISION ON REQUEST FOR RECONSIDERATION OF PATENT TERM ADJUSTMENT AND NOTICE OF INTENT TO ISSUE CERTIFICATE OF CORRECTION."

Patentees request recalculation of the patent term adjustment. Patentees assert, pursuant to 37 CFR 1.703(f), the period of adjustment of the term of the patent under 37 CFR 1.702 is the sum of the periods of examination delay calculated under subparagraphs (a)-(e), to the extent that such periods are not overlapping, less the sum of the periods calculated under 37 CFR 1.704 (the period of applicant delay). Patentees contend no periods of delay attributable to grounds specified under 35 U.S.C. 154(b)(1)(A) and 35 U.S.C. 154(b)(1)(B) overlap. Therefore, patentees maintain that they are entitled to the sum of 322 (299 + 23) days of examination delay plus 380 days of Three Year Delay, reduced by 171 (19 + 61 + 89 + 2) days of applicant delay, for a total patent term adjustment of 531 days. However, in the alternative, patentees assert that in the event that the USPTO maintains its interpretation of 35 U.S.C. 154(b)(1)(B) and 35 USC 154(b)(2)(A), patentees are entitled to 232 days of patent term adjustment, including 23 days of patent term adjustment for the period in excess of four months between payment of the Issue Fee and the date of issuance of the patent.

OPINION

At the outset, the Office reiterates that the period of adjustment under 37 CFR 1.702(b) for failure by the Office to issue the patent within three years is 381 days, not 380 days as stated by patentees. Furthermore, it appears that patentees used the 380 days in calculating the requested patent term determination of 531 days, or in the alternative, 232 days.

Pursuant to 37 CFR 1.703(b), the period of adjustment of 381 day is calculated as the number of days in the period beginning on

the day after the date that is three years after the date on which the application was filed under 35 U.S.C. 111(a), October 24, 2006, and ending on the date the RCE was filed November 8, 2007.[1] "When a period is indicated (in 37 CFR 1.703 or 1.704) as 'beginning' on a particular day, that day is included in the period, in that such day is 'day one' of the period and not 'day zero.'" MPEP 2731. "For example, a period beginning on April 1 and ending on April 10 is ten (and not nine) days in length." Id.

As to patentees' interpretation of the period of overlap, the Office finds that it is inconsistent with the Office's interpretation of the overlap provision, 35 U.S.C. 154(b)(2)(A). 35 U.S.C. 154(b)(2)(A) limits the adjustment of patent term, as follows:

> to the extent that the periods of delay attributable to grounds specified in paragraph (1) overlap, the period of any adjustment granted under this subsection shall not exceed the actual number of days the issuance of the patent was delayed.

As explained in *Explanation of 37 CFR 1.703(f)*[2] *and of the United States Patent and Trademark Office Interpretation of 35 U.S.C. 154(b)(2)(A)*, 69 Fed. Reg. 34283 (June 21, 2004), the Office interprets 35 U.S.C. 154(b)(2)(A) as permitting either patent

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[1] Pursuant to 35 U.S.C. 154(b)(1)(B), 37 CFR 1.702(b) provides, in pertinent part, that:

> Failure to issue a patent within three years of the actual filing date of the application. Subject to the provisions of 35 U.S.C. 154(b) and this subpart, the term of an original patent shall be adjusted if the issuance of the patent was delayed due to the failure of the Office to issue a patent within three years after the date on which the application was filed under 35 U.S.C. 111(a) or the national stage commenced under 35 U.S.C. 371(b) or (f) in an international application, but not including:
>
> (i) any time consumed by continued examination of the application requested by the applicant under section 132(b).

[2] Likewise, 37 CFR 1.703(f) provides that:

> To the extent that periods of delay attributable to the grounds specified in § 1.702 overlap, the period of adjustment granted under this section shall not exceed the actual number of days the issuance of the patent was delayed.

term adjustment under 35 U.S.C. 154(b)(1)(A)(i)-(iv), or patent term adjustment under 35 U.S.C. 154(b)(1)(B), but not as permitting patent term adjustment under both 35 U.S.C. 154(b)(1)(A)(i)-(iv) and 154(b)(1)(B). Accordingly, the Office implements the overlap provision as follows:

> If an application is entitled to an adjustment under 35 U.S.C. 154(b)(1)(B), the entire period during which the application was pending (except for periods excluded under 35 U.S.C. 154(b)(1)(B)(i)-(iii)), and not just the period beginning three years after the actual filing date of the application, is the period of delay under 35 U.S.C. 154(b)(1)(B) in determining whether periods of delay overlap under 35 U.S.C. 154(b)(2)(A). Thus, any days of delay for Office issuance of the patent more than 3 years after the filing date of the application, which overlap with the days of patent term adjustment accorded prior to the issuance of the patent will not result in any additional patent term adjustment. See 35 U.S.C. 154(b)(1)(B), 35 U.S.C. 154(b)(2)(A), and 37 CFR § 1.703(f). See *Changes to Implement Patent Term Adjustment Under Twenty Year Term; Final Rule*, 65 Fed. Reg. 56366 (Sept. 18, 2000). See also *Revision of Patent Term Extension and Patent Term Adjustment Provisions; Final Rule*, 69 Fed. Reg. 21704 (April 22, 2004), 1282 Off. Gaz. Pat. Office 100 (May 18, 2004). See also *Explanation of 37 CFR 1.703(f) and of the United States Patent and Trademark Office Interpretation of 35 U.S.C. 154(b)(2)(A)*, 69 Fed. Reg. 34283 (June 21, 2004).

Further, as stated in the *Explanation of 37 CFR 1.703(f) and of the United States Patent and Trademark Office Interpretation of 35 U.S.C. 154(b)(2)(A)*, the Office has consistently taken the position that if an application is entitled to an adjustment under the three-year pendency provision of 35 U.S.C. 154(b)(1)(B), the entire period during which the application was pending before the Office (except for periods excluded under 35 U.S.C. 154(b)(1)(B)(i)-(iii)), and not just the period beginning three years after the actual filing date of the application, is the relevant period under 35 U.S.C. 154(b)(1)(B) in determining whether periods of delay "overlap" under 35 U.S.C. 154(b)(2)(A).

This interpretation is consistent with the statute. Taken together the statute and rule provide that to the extent that

periods of delay attributable to grounds specified in 35 U.S.C. 154(b)(1) and in corresponding 37 CFR 1.702 overlap, the period of adjustment granted shall not exceed the actual number of days the issuance of the patent was delayed.

It is noted, however, that delays resulting in the Office's failure to meet the time frames specified in 35 U.S.C. 154(b)(1)(A) (the "fourteen-four-four-four" provisions) are not always overlapping with a delay resulting in the Office's failure to issue a patent within the three-year time frame specified in 35 U.S.C. 154(b)(1)(B) because not all application pendency time is counted toward this three-year period. See 35 U.S.C. 154(b)(1)(B)(i)-(iii).

In this instance, all application pendency time is not counted toward the three-year period. A request for continued examination was filed on November 8, 2007. The period subsequent to the filing of the RCE is not included in the three-year time frame specified in 35 U.S.C. 154(b)(1)(B). See 35 U.S.C. 154(b)(1)(B)(i). Thus, the relevant period under 35 U.S.C. 154(b)(1)(B) in determining whether periods of delay "overlap" under 35 U.S.C. 154(b)(2)(A) is the period from October 23, 2003 to November 8, 2007. Thus, only the 299 days[3] of patent term adjustment accorded prior to the filing of the RCE pursuant to 37 CFR 1.702(a)(1)[4] is considered in determining overlap. (The 23 days[5] for Office delay under 37 CFR

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[3] The Office mailed a Restriction Requirement on October 18, 2005, 14 months and 299 days after the filing of the application on October 23, 2003.

[4] 37 CFR 1.702, provides grounds for adjustment of patent term due to examination delay under the Patent Term Guarantee Act of 1999 (original applications, other than designs, filed on or after May 29, 2000).

> (a) Failure to take certain actions within specified time frames. Subject to the provisions of 35 U.S.C. 154(b) and this subpart, the term of an original patent shall be adjusted if the issuance of the patent was delayed due to the failure of the Office to:
> (1) Mail at least one of a notification under 35 U.S.C. 132 or a notice of allowance under 35 U.S.C. 151 not later than fourteen months after the date on which the application was filed under 35 U.S.C. 111(a) or fulfilled the requirements of 35 U.S.C. 371 in an international application[.]

[5] The Office issued the patent on May 12, 2009, 4 months and 23 days after the payment of the issue fee on December 19, 2008.

1.702(a)(4),[6] occurring subsequent to the filing of the RCE is not considered.) The 381 days of Office delay accrued as of the filing of the RCE under 37 CFR 1.702(b) is determined to overlap with the 299 days attributed to examination delay pursuant to 1.702(a)(1). Accordingly, at issuance, the Office entered 82 additional days of patent term adjustment for the Office taking in excess of three year to issue the patent.

In view thereof, the revised determination of patent term adjustment at the time of the issuance of the patent is 233 days (404 days (299 + 23 + 82) of Office delay - 171 days of applicant delay).

The Office acknowledges the previous payment of the $200.00 fee under 37 CFR 1.18(e). No additional fees are required.

This matter is being forwarded to the Certificates of Correction Branch. The Office will issue a certificate of correction indicating that the term of the above-identified patent is extended or adjusted by 233 days.

Telephone inquiries regarding this matter should be directed to the undersigned at (571) 272-3211.

C. T. Donnell

Christina Tartera Donnell
Senior Petitions Attorney
Office of Petitions

Enclosure: Copy of DRAFT Certificate of Correction

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[6] 37 CFR 1.702, provides grounds for adjustment of patent term due to examination delay under the Patent Term Guarantee Act of 1999 (original applications, other than designs, filed on or after May 29, 2000).

> (a) Failure to take certain actions within specified time frames. Subject to the provisions of 35 U.S.C. 154(b) and this subpart, the term of an original patent shall be adjusted if the issuance of the patent was delayed due to the failure of the Office to:
>
> . . .
>
> (4) Issue a patent not later than four months after the date on which the issue fee was paid under 35 U.S.C. 151 and all outstanding requirements were satisfied.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT : 7,531,174 B2

DATED : May 12, 2009

INVENTOR(S) : Sanicola-Nadel et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the cover page,

[*] Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 USC 154(b) by (210) days

Delete the phrase "by 210 days" and insert – by 233 days--

EXHIBIT F

Exhibit F
PTO Delays During Prosecution of U.S. Patent 7,531,174